Vol. XIX. *July, 1910.* *No. 2.*

BIOLOGICAL BULLETIN

THE ASSOCIATION OF A FISH WITH A HYDROID.

HAROLD HEATH.

Among the most interesting phases of animal existence are those examples of commensalism or "messmateism" known in many instances to exist among species of widely different phyla. At times the association, while no doubt beneficial to both parties, is purely accidental, such as that occurring occasionally between the colonies of various hydroids and crabs or molluscs and on two occasions I have found flourishing colonies of *Clava leptostyla* attached to the spines of the sea urchin, *Strongylocentrotus franciscanus*. Again what appears to be a communistic association may in reality be a case of parasitism as, for example, the relation of certain hydroids and the eggs of a number of fishes, or possibly one hydroid to another as noted by Allman in his monograph on gymnoblastic hydroids, or the attachment, mentioned by Fewkes,[1] of the hydroid, *Hydrichthys mirus*, to the fish, *Seriola zonata*. In undoubted cases the association is not only invariable, or fairly constant, but mutually beneficial and very intimate as is witnessed by the fact that the hermit crab, *Eupagurus prideauxii*, when changing its abode removes the commensal anemone, or the case mentioned by Miss Rathbun[2] of the Hawaiian Island crab, *Lybia tesselata*, that held "little sea anemones one in each claw and presented them in a boxing attitude whenever teased or approached by another crab." And, on the other hand, it is generally believed that the anemones enjoy a larger food supply consequent upon the improved method of locomotion.

In 1892 Alcock[3] added what he considered to be another of

[1] *Proc. Boston Nat. Hist. Soc.*, Vol. XXIII.
[2] U. S. F. C. Bulletin, 1903, p. 866.
[3] *Ann. Mag. Nat. Hist.*, 6 ser., Vol. 10.

these remarkable mutual benefit societies, describing the association of the hydroid *Stylactis minoi* with the rock perch, *Minous inermis*. Several specimens were taken at depths varying from forty to seventy fathoms in the Bay of Bengal and the Laccadive or Malabar sea; and in every case the hydroid was found attached in large numbers about the gill opening, on the throat and in the axilla. And not only were no fish of this species ever discovered without being coated with the hydroid, but none of these hydroids was ever found upon the multitudes of other animals dredged in the same locality, though among these were specimens of *Minous coccineus*. Accordingly it thus appears to Alcock that, unusual though it is, this is a case of true commensalism.

Several years later Doflein collected three more specimens of *M. inermis* in Sagami Bay, Japan, and again all were coated, especially between the pectoral and ventral fins, with this same hydroid. In the first account the cœlenterate was assigned by Alcock, on characters associated with the reproductive sacs, to the genus *Podocoryne;* but other specimens, seemingly more highly developed, led the author later to place them in the genus *Stylactis*. On the other hand, Stechow,[1] who described the Japanese specimens, finds no evidence of sporosacs, but young medusæ with tentacles and four radial canals and accordingly this author places the species once more in the genus *Podocoryne*.

During the past summer my friend and colleague, Prof. E. C. Starks, dredged upwards of a hundred specimens of an agonoid fish, *Hypsagonus quadricornis*, in Puget Sound at a depth of approximately forty fathoms. The area over which the dredging extended was in the neighborhood of Friday Harbor and embraced an area of at least two hundred square miles where the bottom varied from sand to mud. Of the 37 specimens preserved in the Stanford University collection 10 of them are coated with a new species of hydroid, *Perigonimus pugetensis*, whose description is given later. In every specimen the cœlenterate was more abundant on the ventral surface of the body, especially in the axilla, and a luxuriant growth was usually found on the pectoral, ventral and, to a less extent, on the anal and caudal fins. With

[1] *Zool. Anz.*, Bd. 32, p. 752, 1908.

one or two exceptions the polyps were much more sparsely distributed over the body and dorsal fins. In no case were they found on the head.

Alcock, referring to several species of fishes of the family Scorpænidæ that "have the body and fins capriciously covered with long, wavy often tufted cutaneous filaments," believes, with a large company of zoologists, that these structures "assist in giving the fish a deceitful resemblance to the incrusted rocks of its environment, in order to allure, or at any rate not to scare, prey. And it appears probable that *Stylactis minoi* enables its companion *Minous inermis* in the same way to assume the same convenient and successful disguise." While the evidence is

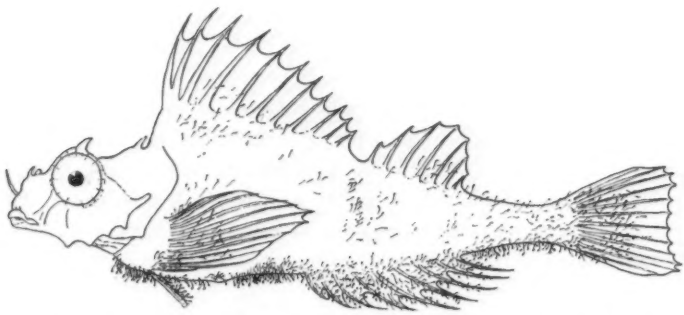

FIG. 1. An agonoid fish (*Hypsagonus quadricornis*) bearing a hydroid colony (*Perigonimus pugetensis*). Natural size.

strong that these devices do enable their possessor to escape detection and wage more successfully its battle for existence, and while the hydroid may enable the fish in question to more closely harmonize with its surroundings it does not follow even then that this is a case of commensalism. Nevertheless, as Hickson points out,[1] the fact that "the fish is never found without this hydroid, nor the hydroid without this species of fish, suggests very strongly that there is a mutual advantage in the association."

In the present case the evidence is not so cogent. About one fourth of the fishes only were overgrown with the hydroid and

[1] "Camb. Nat. Hist.," Vol. I., p. 268.

other specimens taken by the U. S. F. C. Str. "Albatross" in the open ocean off the Washington coast and in Bering Sea, are totally without them. These last named specimens, coming from the same depth (40 fm.) occurred on a pebbly bottom or one of broken shell and it is possible that the Puget Sound individuals, without the cœlenterate, occurred in a similar habitat. Be that as it may, it is a suggestive fact that in the fishes under consideration the hydroid was "attached in large numbers about the gill opening, on the throat and in the axilla," in other words over the ventral surface that is already the most concealed portion of the body. Referring to *Hypsagonus quadricornis* Prof. C. H. Gilbert writes in Jordan and Evermann's "Fishes of North and Middle America" (p. 204): "In the aquarium the fish appears to walk, resting alternately on the upper and lower pectoral rays and on the front rays of the anal." Under such circumstances the eddies produced in the bottom ooze would naturally bring the greatest amount of organic material to animals ventrally situated. The appearance strongly suggests that the advantage lies rather with the hydroid just as it does with the several species of barnacles attached to the skin of the whale. Whether the association is any more intimate in the case Alcock cites it is impossible to state conclusively, but the evidence is certainly not entirely convincing.[1]

Prof. C. C. Nutting, to whom I have submitted specimens, has kindly identified them as a species of *Perigonimus*, its nearest relative being apparently *P. vestitus* Allman. As in other members of the genus the hydrorhiza forms a highly branched, frequently anastomosing, system over the surface of the fish, but so far as noticed this contact is purely superficial, there being no evidence of parasitism. And furthermore the presence of small entomostracans and nondescript organic remains in the gastric

[1]Since this paper was sent to press I have examined upwards of two dozen specimens of this same species of rock perch (*M. inermis*) collected by my colleague Prof. J. O. Snyder, at Onomichi, on the Inland Sea, in the Province of Bingo, Japan. All of these are excellently preserved and in no instance has a hydroid been found upon them. It thus becomes more certain that the association described by Allcock is not an undoubted case of commensalism. Professor Snyder has called my attention to the fact that according to Regan (Ann. and Mag. Nat. Hist., 1905, Vol. XV., p. 20) *Minous inermis* should be *Minous monodactylus* (Bloch and Schneider).

cavity of the hydranths shows the feeding processes to be those of a non-parasitic species.

At frequent intervals branches, 3 to 4 mm. in height when fully developed, spring from this root system and each is terminated by a single hydranth. In no case does a hydranth arise as a lateral bud from the hydrocaulus, as in *P. vestitus*, for example. On the other hand, the medusa buds almost invariably appear as isolated, very rarely closely associated pairs of outgrowths dis-

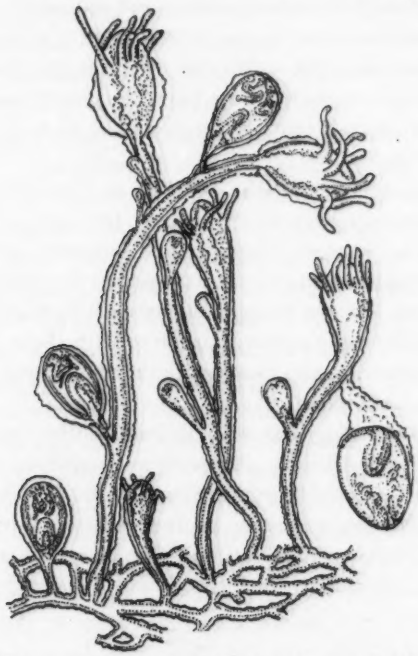

FIG. 2. Portion of hydroid colony (*P. pugetensis*).

posed at comparatively regular intervals along the stem. Their order of appearance is seemingly not so definite, though this, in part at least, is perhaps due to the escape of an unknown number of medusæ from the older stems. On the shorter, younger branches one bud appears usually in the vicinity of the base or the hydranth, and about the time its development is half way completed a second one arises in the middle section of the stem,

while a third frequently makes its appearance in the vicinity of the hydrorhiza about the time the first medusa is liberated. Beyond this point the order of development is not known, but Fig. 2 illustrates a few of several different stages. The mode of development of the medusa is typical, and results in a bi-tentaculate type.

With the exception of the distal portion of each hydranth, including the tentacles, the entire colony is ensheathed in a cuticle often coated with minute organisms and sediment. In the older portions this investment is comparatively firm with the exception of that surrounding the hydranths, which is less dense and more flexible. The medusa buds are likewise covered, and for a time prior to their detachment are bound to the stem by an irregular cuticular bridge.

In the younger hydranths the line of demarcation between them and the stem is not clearly defined, but as they become older the boundary is more distinct, the hydranth growing more globular owing, to some extent at least, to the greater height of the endoderm. In the younger stages each hydranth bears four tentacles, later four others appear, often with slight irregularities in the time intervals, and finally with the appearance of four more the number is complete.

The following diagnosis will distinguish the present species from other known forms: *Perigonimus pugetensis* new species, twelve tentacles. Hydranths arising invariably from the hydrorhiza, and bearing as many as four scattered bi-tentaculate medusæ. Cuticle relatively thin. Occurs on the agonoid fish, *Hypsagonus quadricornis*, in Puget Sound, Washington.

THE CHROMOSOMES IN THE OÖGENESIS, FERTILIZATION AND CLEAVAGE OF COREID HEMIPTERA.

CHARLES V. MORRILL.

I. Introduction.

The recent conclusions regarding sex-production based on the nuclear dimorphism of the spermatozoa in the tracheates have involved certain assumptions regarding oögenesis and cleavage which, though made in conformity with many well-determined facts, are still in need of more adequate support from direct observations. The principal of these assumptions is that in the formation of the polar bodies, the diploid chromosome-groups of the female are reduced to haploid groups that are alike in all the mature eggs. Further, since the spermatozoa are of two sorts, the embryos produced would be correspondingly different and this difference should be apparent from a study of the embryonic nuclei. At present, however, the complete chromosome-cycle has been worked out in only two groups of tracheates, the phylloxerans and aphids. In the phylloxerans, Morgan ('08, '09) has traced the full history of the chromosomes through several generations and the combined observations of Stevens ('05*a*, '06*a*, 09) von Baehr ('08, '09) and Morgan ('09) practically completes the cycle in the aphids. The very recent observations of Boveri and Gulick ('09), though as yet published only in brief outline, show that in *Heterakis*, a nematode, the chromosome-cycle is similar to that assumed for many insects and bears the same relation to sex-production, while Baltzer ('08) has found that in sea-urchins, the conditions are the reverse of those in insects, the eggs being dimorphic and the spermatozoa all of one kind.

The present work[1] was undertaken with the hope of demonstrat-

[1]This problem was begun in the zoölogical laboratory of Columbia University and completed in the anatomical laboratory of the College of Medicine, Syracuse University. A part of the material was collected while occupying a John D. Jones Scholarship room at the biological laboratory, Cold Spring Harbor, N. Y., during the summer of 1907, and a Wistar Institute room at the Marine Biological Labor

ing the chromosome groups in the oögenesis, fertilization and cleavage in certain coreid hemiptera and of determining in this way, if possible, whether the assumptions made in regard to the number and behavior of the chromosomes in these stages is in accordance with the facts.

Four species of the Coreidæ were examined: *Archimerus alternatus* Say, *Anasa tristis* De Geer, *Protenor belfragei* Hagl., *Chelinidea vittigera* Uhler.[2] In all of these forms the spermatogonia have been found to contain an odd number of chromosomes, one of which (the unpaired idiochromosome[3]) fails to divide in one of the maturation divisions. One half the spermatozoa thus contain this chromosome, the other half lack it, and a dimorphism of the spermatozoa arises. The oögonia have been shown to have an even number of chromosomes, the unpaired element of the spermatogonia being represented here by a pair of equal size. The maturation of the egg had not been fully worked out, but it was assumed that every chromosome divides equally in both polar divisions, giving to the mature egg a group of chromosomes similar to that borne by a spermatozoön having the idiochromosome. The eggs were accordingly assumed to be all of one kind with respect to their chromatin content, as direct observation has shown to be true in phylloxerans (Morgan), aphids (Stevens, von Baehr) and more recently in *Heterakis* (Boveri and Gulick.)

atory, Woods Hole, Mass., in 1909. To the directors of these laboratories I am indebted for the facilities placed at my disposal. I wish also to express my gratitude to Professors E. B. Wilson and T. H. Morgan for the many helpful suggestions they have made during the progress of this work.

[2]I am indebted to Mr. E. P. Van Duzee, of Buffalo, N. Y., for the identification of my material. The species *Archimerus alternatus* is almost, if not quite, identical with the *A. calcarator* of Professor Wilson's material (identified by Mr. P. R. Uhler) and was collected from the same locality in Van Cortlandt Park, New York. *Protenor* was found at Woods Hole, Mass., and *Anasa* at Woods Hole, and Cold Spring Harbor, N. Y. *Chelinidea* was taken by Professor Wilson at Southern Pines, N. C. A part of the living specimens of *Anasa* were also furnished by Professor Wilson

[3]For the sake of simplicity, the term "idiochromosomes" will be used in this paper to designate those chromosomes which are associated with sex-production, irrespective of their detailed behavior in the growth periods or maturation divisions. It will thus include the "idiochromosomes" (in the more restricted sense), "accessory," "odd" or "heterotropic" chromosomes, "monosomes," "heterochromosomes," "X- and Y-elements," etc., of recent writers. Professor Wilson has used this term in a similar sense in the fourth of his "Studies" ('09*b*).

It was further supposed that if an egg is fertilized by a spermatozoön bearing the idiochromosome an embryo will result whose nuclei all have an even number of chromosomes, similar to the oögonial groups, but if fertilized by a spermatozoön lacking that chromosome, the embryonic nuclei will have an odd number of chromosomes similar to the spermatogonial groups. Accordingly the former will be females, the latter males. It should be possible, then, to distinguish the sex of an embryo by an inspection of the embryonic (somatic) chromosome groups. Again, if the number of chromosomes in the male and female pronuclei could be accurately determined just before the first cleavage spindle is formed, it would afford additional evidence of the dimorphism of the spermatozoa and the relation this condition bears to sex-production.

II. Material and Methods.

The insects were brought into the laboratory or greenhouse and placed in cages in which their food plants were growing. Here they paired readily and laid their eggs either on the plants or on the sides or bottom of the cages. The breeding periods of the four genera employed differ widely. *Anasa* may be found pairing on squash plants in the vicinity of New York or Woods Hole early in July, the eggs being laid in clusters on the under surfaces of the leaves, but specimens kept in a greenhouse over the winter laid early in May. *Chelinidea*, brought from the south and kept in a greenhouse during the winter, began to lay its clusters of eggs in the latter part of March. *Archimerus* begins laying on the goldenrod in the vicinity of New York in the latter part of May or first of June. The eggs are laid singly, and it was found impossible to collect sufficient numbers in the field so that all those used were taken from caged individuals. *Protenor* also lays its eggs one at a time and, in the laboratory, rarely makes any attempt at fastening them to any object, but drops them to the bottom of the cage where they were collected in small quantities. In addition to these, a number of eggs were taken from the oviducts of *Anasa* and *Protenor*.

The eggs of the four species differ markedly in size, *Archimerus* having the largest and *Protenor* the smallest, those of *Anasa* and

Chelinidea being intermediate; these size differences correspond roughly with the difference in size of the several species. All the eggs, whether in the oviduct or after laying, are enclosed in a tough brown chorion.

Several different fixing fluids were tried. Flemming's strong fluid, Gilson's mercuro-nitric and Bouin's picroformol were found very uncertain in result, as they seldom penetrate the thick chorion. All these can be used, however, if the eggs are pricked with fine needles before placing them in the fixing fluid, but their action is such as to render the yolk very brittle and difficult to cut. By far the best results were obtained by placing the eggs immediately in the Gilson-Carnoy acetic-alcohol-chloroform-sublimate mixture for fifteen to thirty minutes or in a mixture of glacial acetic, one part, absolute alcohol saturated with sublimate, two parts, for five to ten minutes. After either fluid, the eggs were transferred to iodized 95 per cent. alcohol for twelve hours and preserved in 80 per cent. alcohol. The acetic-alcohol-sublimate mixture was found invaluable for the earliest stages of maturation which occur while the eggs are still in the lower part of the oviduct and directly after laying. For later stages, the Gilson-Carnoy mixture gave excellent results. After immersion in alcohol, the egg shrinks away from the chorion which can then be removed with fine forceps and cutting needle. After removing the chorion, the eggs were dehydrated, cleared in cedar oil and immersed in melted paraffin for two hours. They were then oriented in a drop of paraffin and embedded. Serial sections were cut 6-8 μ thick on a sliding microtome. Very good series can be obtained in this way though the yolk sometimes becomes brittle and troublesome. The stain most frequently employed was iron-hæmatoxylin with or without a counter-stain. In addition to the eggs, ovaries and testes were fixed in Flemming's strong fluid and stained in iron-hæmatoxylin or safranin.

About twelve hundred eggs in all were sectioned, but owing to mechanical difficulties in technique, only about two hundred of these were of any value for study. In the maturation and fertilization stages particularly, one or two poor sections may render an entire series worthless, though in later embryonic stages this difficulty is not so serious. For this reason the results are

necessarily somewhat meagre, but they are perfectly clear as far as they go.

III. Descriptive.

A. *Oögenesis.*

The results on oögenesis are confined to the chromosomes of the oögonial and oöcyte divisions. No attempt has been made to trace the full history of the growth period, but an examination of a few eggs taken from the ovarian tubes seems to show that no definite chromatin-nucleolus or persistent oögonial chromatin element is present, as stated by Wilson ('06). The nucleus at this time contains many faintly staining chromatin threads and several small nucleoli whose nature was not determined. Foot and Strobell ('09) have found the same condition to be true in *Euschistus*, a pentatomid.[4]

1. *Archimerus alternatus.*

The spermatogonial groups[5] have been figured by Wilson in the second of his "Studies" ('05*b*), but for the sake of comparison two more are shown (Fig. 1, *c* and *d*). Each group has 15 chromosomes, two of which, the *m*-chromosomes (following Wilson's terminology), can always be identified by their very small size. Of the remaining thirteen no one can be positively identified, by its size or shape, as the idiochromosome. In the spermatocyte divisions this chromosome passes undivided to one pole of the spindle in the *first* mitosis and divides equally in the second (Wilson, '05*b*). This condition is peculiar to *Archimerus* alone of all the Coreidæ so far described,[6] but a rëexamination of the spermatocyte stages in new material shows beyond doubt that Wilson's account is correct. The idiochromosome can be easily identified in the first mitosis by its peripheral position on the spindle and by its unconstricted contour when the other chromosomes are in early, and even late, anaphase. It passes to one pole of the spindle a little behind the others. Further, in the

[4]Stevens ('06*b*) has described "heterochromosomes" in certain stages of the growth period of the oöcytes of *Aphrophora*, an homopteran.

[5]See footnote 2, at bottom of page 80.

[6]Professor Wilson has also found a similar condition in *Pachylis gigas* (unpublished).

cysts of second spermatocytes, one finds metaphase groups with eight and seven chromosomes side by side.

The oögonial groups[7] have not been figured previously, but Wilson gives the number of chromosomes as 16 in the fourth of his "Studies" (09*b*). In Fig. 1, *a* and *b*, two plates are shown

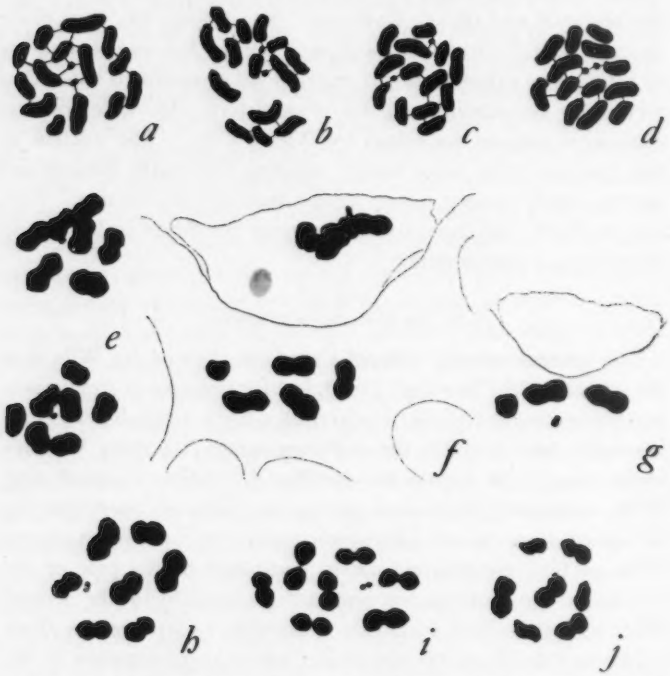

FIG. 1. *Archimerus alternatus*. *a* and *b*, oögonial, *c* and *d*, spermatogonial metaphase-groups. *e–j*, first oöcyte-(polar) division; *e*, daughter groups from the same anaphase-spindle, polar view; *f* and *g*, anaphase, side view, in two sections; *h* and *i*, inner daughter groups, polar view, of two anaphases; *j*, outer daughter group, polar view, of an anaphase.

each with 16 chromosomes. A pair of *m*-chromosomes again appears in each group. The fourteen larger elements present a somewhat graded series and cannot be paired off readily. Two

[7]The unreduced female groups in all the species, were taken from the region in the ovary lying just below the nutritive chamber, where oögonia, follicle-cells and young oöcytes are found together. For convenience they will be called "oögonia."

of the largest are probably the idiochromosomes, though they do not differ sufficiently from the others, in size or shape, to admit of exact identification.

The anaphase of the first mitosis was the earliest oöcyte stage obtained and was found in eggs just after laying. Fig. 1, *e* (from two sections), shows a polar view of this stage in which eight chromosomes can be accurately counted in both outer and inner groups. There are seven large dyads and one very small one in each, corresponding in relative size to the fourteen large and two small chromosomes of the oögonia. Fig. 1, *f* and *g*, show a side view of a late anaphase from two sections. The inner group contains eight dyads; one of them has divided prematurely, the two parts appearing in neighboring sections. The chromosomes in the outer group are too crowded to be counted. Pl. I., *a*, shows another anaphase in which the inner group is complete in one section and contains eight dyads. Fig. 1, *h* and *i*, show the inner groups of two more anaphases, polar view. The chromosomes in both are all dyads, eight in each, the component parts of which show all degrees of separation. This premature division of the dyads is very common in the final anaphases of the first division and might lead to mistakes in counting if it were not that one finds all stages of division up to the complete separation shown in Fig. 1, *f* and *g*. Henking found this condition in the egg of *Pyrrhocoris* ('92, Pl. III.) and it has also been found in the first spermatocytes of *Aphrophora* an homopteran (Stevens, '06*b*) and *Anax* a dragon-fly (Lefevre and McGill, '08). It is probable that even in cases of extreme separation, the halves of the dyads remained connected by fine strands of chromatin or linin which become invisible after long extraction of the stain. The second division is thus foreshadowed, in the anaphase of the first, and even before as will be shown in *Anasa*. Since there is no period between the first and second divisions when the chromosomes lose their individual contour, in fact no telophase in the strict sense, the dyads pass practically unchanged into the second maturation spindle.

The chromosomes which enter the first polar body retain their contour and grouping for some time, forming a flat plate when seen in surface view. Fig. 1, *j*, shows such a plate with seven

large chromosomes all more or less constricted and a small nodule close to the central one, which probably is the *m*-chromosome. Fig. 2, *b*, shows another in somewhat oblique view with seven large dyads, and the *m*-chromosome dyad, the latter faintly stained. Fig. 1, *f*, and Pl. I., *a*, show side views of two more polar body groups; in Fig. 1, *f*, the *m*-chromosome dyad is dis-

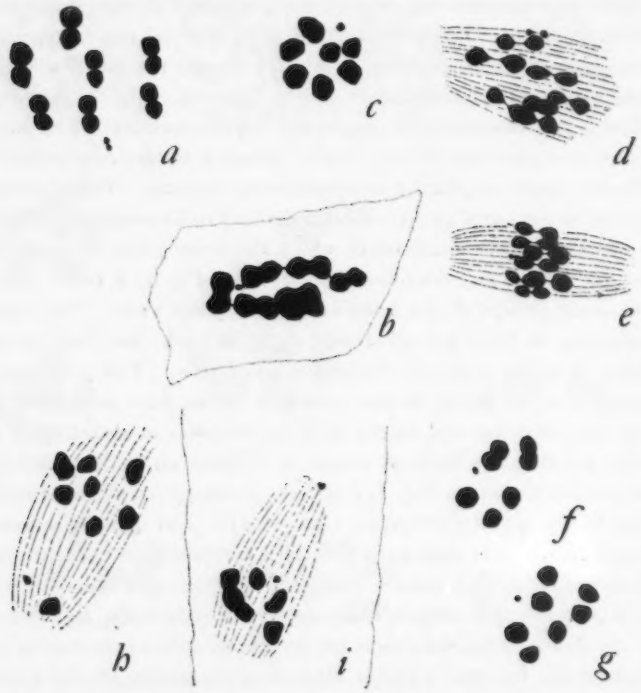

FIG. 2. *Archimerus alternatus*. Second oöcyte-(polar) division. *a*, metaphase-group, oblique view. *b*, chromosome-group of the first polar body from the same egg as the last. *c*, metaphase-group, polar view. *d* and *e*, metaphase groups, side view. *f* and *g*, daughter groups from the same anaphase-spindle, oblique view. *h* and *i*, anaphase, oblique view, in two sections.

tinctly seen. All the chromosomes show the typical constriction as though ready for a second division. However no spindle is formed and no division takes place except that in individual chromosomes the halves of a dyad may separate of their own accord,

just as in those of the inner groups. At the close of the second division the chromosomes of the first polar body are finally merged together in a deeply-staining mass.

The second division follows closely upon the first as stated above. The chromosomes do not crowd together in the final anaphase of the first division as in the spermatocytes, but merely rotate about forty-five degrees and become disposed upon a new spindle which forms out of the enveloping cytoplasm. Fig. 2, *a*, shows a metaphase of the second division in slightly oblique polar view. The seven large dyads and *m*-chromosome dyad are sharply constricted and ready for division. The first polar body with its eight dyads taken from the same section is shown in Fig. 2, *b*. Fig. 2, *c*, shows another polar view of a second division metaphase, and Fig. 2, *d* and *e*, are side views of the same, each showing eight chromosomes. All the dyads are more or less drawn out in preparation for division, and in anaphase, separate into two groups of monads. Fig. 2, *f* and *g*, show outer and inner groups respectively taken from the same anaphase; each has eight monads. Fig. 2, *h* and *i*, show an oblique view of an anaphase in two sections (the surface of the egg is indicated by a dotted line at the right of each section). The outer group which passes into the second polar body is shown above, the seven large monads in *h*, the *m*-chromosome monad in *i*. The inner group which remains in the egg, is shown below; six of the large monads and the *m*-chromosome monad in *i*, the remaining large monad in *h* (The stippled object in *h* is part of one of the chromosomes in *i*). Thus the end result is, that the female pronucleus is formed from eight single elements or monads, comparable to those borne by a spermatozoön containing the idiochromosome (vid. Wilson, '06).

There are no peculiar or "lagging" chromosomes in either division. Which of the polar divisions is reducing, *i. e.*, separates whole chromosomes which have paired in synapsis, could not be determined, since the first oöcyte prophases were not obtained.

While it is not possible to identify the idiochromosomes in the two divisions just described, the *m*-chromosome appears as a constant element dividing in both. In the spermatocytes Wil-

son's ('05*b*) figures show it occupying the centre of spindle in both divisions. However, in the oöcytes its position is variable in the first division (Fig. 1, *e*, *h*, and *i*; Pl. I., *a*) but always peripheral in the second (Fig. 2, *a*, *c*, *d*, *f* and *g*, *h* and *i*).

FIG. 3. *Anasa tristis*. *a* and *b*, oögonial, *c* and *d*, spermatogonial metaphase-groups. *e–j*, first oöcyte-(polar) division: *e*, *f*, *g* and *h*, metaphase-groups, polar view. *i* and *j*, daughter groups from the same anaphase-spindle, polar view.

2. *Anasa tristis*.

The spermatogonial groups have been figured by Wilson ('05*b* and '06), and the number of chromosomes stated as 21. Wilson's count has been corroborated by Montgomery ('06) and by Lefevre and McGill ('08) though Paulmier ('99) and Montgomery in an earlier paper ('01) had given the number as 22. Foot and Strobell ('07*a* and 07*b*) have disputed the twenty-one count and, on the basis of photographs of smear preparations, have concluded that the number is 22, confirming Paulmier's original account. A reëxamination of fresh material from two different localities gave 21 as the correct number of spermatogonial chromosomes in agreement with accounts of Wilson, Montgomery, and of Lefevre and McGill. Imperfect plates it is true may show less than this number, but none were found with more. In *Chelinidea vittigera*, where the spermatogonial groups are almost identical with those of *Anasa*, a single exception may be cited, in which 22 chromosomes were found. Apart from this, all clear plates gave 21.

As pointed out by Wilson and by Lefevre and McGill, the spermatogonial groups of *Anasa* show two *m*-chromosomes and three largest chromosomes one of which must be the unpaired chromosome ("heterotropic" or "accessory" chromosome) though it cannot be precisely identified by its size or shape. Fig. 3, *c* and *d*, shows two spermatogonial groups illustrating these points.

The oögonial groups (Fig. 3, *a* and *b*) contain 22 chromosomes, including two *m*-chromosomes and four largest chromosomes. Of the last named, two must be the idiochromosome pair, corresponding to the unpaired idiochromosome of the spermatogonia, as pointed out by Wilson in the third of his "Studies" ('06).

The metaphase of the first oöcyte division was found in eggs taken from the lower part of the oviduct. Fig. 3, *e*, *f*, *g* and *h*, shows four such phases, *h* being a composite figure from two sections.[8] There are 11 chromosomes in each, all undoubtedly

[8]The difference in size between the chromosomes as a whole of Fig. 3, *e* and *f*, and those of Fig. 3, *g* and *h*, is probably due to a difference in the length of fixation. In the former case the eggs were left for ten minutes in the fixative (alcohol-acetic-sublimate), in the latter, for five minutes. The chromosomes of Fig. 3, *g* and *h*, seem to show the swelling action of the acetic unchecked by the sublimate which penetrates more slowly.

FIG. 4. *Anasa tristis*. *a–f*, first oöcyte-(polar) division; *a*, four chromosomes in initial anaphase, side view, showing tetrad-character; *b*, final anaphase, side view; *c* and *d*, daughter groups from the same anaphase-spindle, slightly oblique polar view; *e* and *f*, the same from another egg; *g*, metaphase-group, side view, second oöcyte-(polar) division. *h*, the chromosomes of the last drawn at three different focal levels. *i*, the chromosome-group of the first polar body from the same eggs

double, arranged in an irregular ring with one in the centre and one or two outside. Unlike the first spermatocytes, the central chromosome is not the *m*-chromosome bivalent but one of the larger ones, while the *m*-chromosome itself lies just within the ring (Fig. 3, *e*, *f* and *h*) or somewhat outside (Fig. 3, *g*). In these four metaphases, the axis of the spindle is somewhat oblique to the plane of section and the structure of the chromosomes can thus be readily observed. As might be expected many have the shape of typical tetrads foreshadowing the two oöcyte divisions. This is especially well seen in Fig. 3, *e*, where even the *m*-chromosome has the quadripartite form. Whether distinctly quadripartite, or simply dumb-bell shaped, the plane of the first division is clearly indicated in every chromosome. In Fig. 3, *e* and *h*, there are two bivalents (*M* and *N*) perceptibly larger than any of the others. In all probability these arise from a pairing of the four largest chromosomes of the oögonia. Accordingly one of them may be considered to be the idiochromosome bivalent.

The anaphase of the first polar spindle was found in eggs directly after laying.[9] The chromosomes divide in the plane already indicated at metaphase. Fig. 4, *a*, shows a side view of four chromosomes in initial anaphase; the tetrad character of each is clearly indicated. Fig. 3, *i* and *j*, shows polar views of the outer and inner groups respectively from the same spindle; each has 11 chromosomes. All, with the exception of the *m*-chromosome in *j*, are obviously dyads showing all degrees of constriction, as in the first polar anaphase of *Archimerus*. The outer group (*i*) which passes into the first polar body shows two dyads completely divided though the polar body itself neither divides nor forms a spindle. In both groups two largest dyads (*M* and *N*) can be distinguished. Fig. 4, *c* and *d*, are outer and inner groups respectively from another spindle, in slightly oblique polar view (in *c* the large chromosome, *N*, was found in the same section as the inner group, *d*). Both groups contain 11 dyads of which two (*M* and *N*) are larger than any of the others. Fig. 4, *e* and *f*, are outer and inner groups respectively of a third anaphase in

[9] A single exception to this was found. One egg taken from the lowest part of the oviduct showed the first polar spindle in initial anaphase. It is possible that rough handling started the maturation process, as has been found in other groups of animals.

somewhat oblique polar view (the entire outer group, *e*, and six chromosomes of the inner group, *f*, appeared in one section, the remaining five of the latter group, in the next section). Both groups contain 11 dyads of which several in *f* show premature separation of their parts.

The peripheral position assumed by the *m*-chromosomes in metaphase (Fig. 3, *e*, *f* and *g*) is again seen in all the anaphase groups (Fig. 3, *i* and *j*; Fig. 4, *c*, *d*, *e* and *f*). This is undoubtedly their normal position in the first polar division. A side view of the final anaphase (Fig. 4, *b*) shows no peculiar or "lagging" chromosome on the spindle. All the chromosomes divide equally, as the polar views of three final anaphases show, where every chromosome is distinctly visible.

A single example of the second polar division was found (Fig. 4 *g*). It is a side view of a metaphase with 11 dyads. In *h*, the dyads of this group are drawn at three different focal levels. The first polar body (*i*) of the same egg also contains eleven dyads. In both the second polar spindle and the first polar body, there are two dyads (*M* and *N*) somewhat larger than any of the others. These are undoubtedly the products of division of the two largest tetrads of the first polar metaphase (Fig. 3, *e* and *h*). The *m*-chromosome in the second division (Fig. 4, *g* and *h*) takes a peripheral position as it did in the first. It appears also in the first polar body (*i*). In short there are three chromosomes distinguishable by their size, which can be identified in both polar divisions. These in all probability arise from a pairing of the four largest and two smallest oögonial chromosomes.

Though no anaphases of the second division were found, it is almost certain from the results in *Archimerus* that all the dyads divide equally, in a plane corresponding to the constriction shown at metaphase. Accordingly the female pronucleus, as well as the second polar body, will contain 11 monads and correspond to a spermatozoön bearing the idiochromosome as assumed by Wilson ('06).

3. *Protenor belfragei.*

The spermatogonial groups have been described and figured by Montgomery ('01 and '06) and by Wilson ('06). These observers agree that there are 13 chromosomes, one of which is

more than twice as large as the next in size. Of the remaining twelve, two are much larger than the others, and the rest form a graded series of pairs. The smallest pair, the *m*-chromosome, is relatively larger in this species than in *Archimerus* and *Anasa*. In Fig. 5, *c* and *d*, are shown two spermatogonial groups illus-

FIG. 5. *Protenor belfragei*. *a* and *b*, oögonial, *c* and *d*, spermatogonial metaphase-groups. *e–h*, first oöcyte-(polar) division; *e*, metaphase group, polar view; *f*, metaphase group, oblique polar view; *g*, six chromosomes in metaphase, side view; *h*, five chromosomes in metaphase, side view.

trating the above mentioned points. The idiochromosome does not appear constricted as figured by Montgomery ('01), Fig. 134.

The oögonial groups were figured by Wilson in the third of his "Studies" ('06). In them "there are two very large chromosomes, equal in size, in place of the single one that appears in the male, while the remaining chromosomes show the same relations as in the male." In Fig. 5, *a* and *b*, two of these groups are shown.

Of the maturation stages, only four preparations were obtained,

all of the first polar metaphase (Fig. 5, *e*, *f*, *g* and *h*).[10] Two of these (*e* and *f*) are complete, showing seven bivalents. The idiochromosome bivalent can be positively identified by its relatively enormous size, having been formed, no doubt, by the synapsis of the two largest chromosomes (idiochromosomes) of the oögonia. It does not in any way resemble a nucleolus. The bivalent next in size, corresponding to the two next largest chromosomes of the oögonia, can also be identified. Indeed the bivalents as a whole show the same relative size differences as the chromosome pairs in the oögonia. The *m*-chromosome bivalent is the smallest but only slightly smaller than the next in size. In Fig. 5, *f*, three chromosomes of intermediate size are seen in face view; two of these exhibit a quadripartite form, clearly indicating their bivalent nature. Fig. 5, *g*, is a side view of an incomplete metaphase, drawn from two sections, showing six of the seven chromosomes, and Fig. 5, *h*, shows a metaphase, side view with only five chromosomes, again taken from two sections. In both of these figures the idiochromosome bivalent can be readily identified by its size and the plane of the second division is clearly indicated in all the bivalents. While the further processes of maturation were not followed, it may be inferred, on the analogy of *Archimerus*, that in the anaphase of the first division the seven tetrads divide into two groups of dyads and the inner group of these separate in the second division into two groups of monads; the inner group of these last named, seven in number, enter into the formation of the female pronucleus, which is thus similar in chromatin-content to a spermatozoön bearing the idiochromosome.

4. *Conclusions Regarding Oögenesis.*

The results on maturation are somewhat meagre it is true but perfectly clear as far as they go, and point to the conclusion that, unlike the spermatozoa, all the mature eggs are of one kind with respect to their chromatin-content, as has been assumed. The female pronucleus contains a reduced group of chromosomes similar in size and number to that carried by a "female-pro-

[10]The excessive size of the chromosomes in these figures, especially those of *g* and *h*, is probably due to the peculiar action of the fixative (see footnote 8, on page 89).

ducing" spermatozoön, *i. e.*, one bearing the idiochromosome (directly proved in *Archimerus*, but only in part in *Anasa* and *Protenor*). The idiochromosome bivalent is not distinguishable by its behavior from other chromosomes, and divides in both mitoses, giving an equal portion to each oötid. It never assumes a nucleolus-like form either in the oögonial or oöcyte divisions.

B. *Some Details of Polar Body Formation.*

The place of polar body formation has been found to vary in different groups of insects. It may be on the dorsal surface, either approximately midway between the poles as in *Blatta* (Blochmann, Wheeler) and *Pyrrhocoris* (Henking), or a short distance behind the anterior end as in *Musca* (Blochmann, Henking). In Chrysomelidæ (Hegner) it is ventral, while in *Pieris* (Henking) it is close to the anterior (micropylar) end. In *Hydrophilus* (Heider) and *Aphis* (Stevens) it is lateral. In *Archimerus* the polar bodies are given off on one of the flat surfaces of the egg, approximately midway between the poles. In this species the two surfaces cannot be distinguished after the chorion has been removed, but in *Protenor* the dorsal surface is markedly convex while the ventral is flat or slightly concave, and it was here determined by proper orientation that the place of polar body formation is on the dorsal surface. The first polar spindle lies in a small thickening of cytoplasm with its axis at right angles to the egg surface (Fig. 1, *f* and *g*; Fig. 4, *a* and *b*; Pl. I., *a*). The anaphase of the first polar division occurs just after laying (in *Archimerus* a single exception was found; see footnote 9, page 91). No centrosomes or asters could be demonstrated by the method of fixation used just as Henking ('92) found in *Pyrrhocoris* though he used a different method. In late anaphase a cell-plate is formed by swelling of the spindle fibers and the surface of the egg dips down and around the outer group of chromosomes, until finally a little mass of cytoplasm containing the latter comes to lie free in a depression of the egg surface (Fig. 1, *f* and *g*; Fig. 4, *b*; Pl. I., *a*). In both the first and the second polar divisions (Fig. 4, *b*, and Pl. I., *b*) the constriction probably does not involve the cell-plate but passes between it and the outer group of chromosomes as Henking observed in *Pyrrhocoris*.

In *Anasa*, a rounded cytoplasmic body was found, in two cases, in or near the first polar spindle (Fig. 3, *i*; Fig. 4, *c-d*). This is perhaps comparable to the "eigenthümliches Körperchen" which Henking described in *Pyrrhocoris*. It may be a plasmosome, but it is difficult to decide, since one frequently finds a number of cytoplasmic bodies in the neighborhood of the first polar spindle (Pl. I., *a*) which cannot be distinguished from yolk granules and which are inconstant in appearance and number.

At the conclusion of the first polar division the spindle gradually fades away; there is no persistent cylindrical mass of spindle fibers or "thelyid" as Henking ('90 and '92) found in *Pieris*, a lepidopteran, and *Agelastica*, a coleopteran. The chromosomes left in the egg, as stated before, remain separate and there is no telophase in the strict sense. After a short resting period, they rotate about 45° and become disposed on a new spindle which has formed out of the cytoplasm surrounding them. The axis of the second polar spindle lies very obliquely to the surface of the egg (Fig. 2, *h* and *i*; Pl. I., *b*). As in the first division there are no centrosomes or asters. In late anaphase a cell-plate is formed by swellings of the spindle fibers. The second polar body is constricted off in the same manner as the first and lies alongside of it in the same depression. The first does not divide. The two bodies finally become embedded in the surface cytoplasm and can be distinguished as late as the third or fourth cleavage.

At the close of the second polar division, the chromosomes left in the egg become massed together and are converted into the female pronucleus (Pl. I., *b*). Those which have entered the polar bodies may remain separate for some time but eventually fuse into one or two deeply staining masses.

C. *Fertilization.*

The spermatozoa enter the egg through the micropyles which form a conspicuous ring at the anterior end. Polyspermy is undoubtedly normal, for accessory sperm nuclei were found in the egg as late as the copulation stage shown in Pl. II., *b*. As many as three of these nuclei appeared in some cases. At the time when the first polar spindle is in late anaphase, the sperm head enveloped in a mass of cytoplasm has moved some distance

into the egg among the yolk spheres leaving a train of cytoplasm behind it. It appears as a compact deeply staining rod surrounded by a clear area and preceded by an aster (Pl. I., *d*). The clear area is probably the "arrhenoid" mentioned by Henking ('92) in his account of *Pyrrhocoris*. The sperm head often appears coiled at the end which points away from the direction of its movement. Later it loses its staining power and opens out into an oval vesicle (Pl. II., *a*). The clear area and aster are here well marked though no centrosome is visible in the preparation. Still later, the vesicle becomes considerably larger and small irregular masses of chromatin can be seen in its interior. It is then ready for copulation.

In the meantime, the egg nucleus, formed from the inner group of chromosomes of the second polar spindle, has begun to move into the egg surrounded by a small mass of cytoplasm (Pl. I., *c*). The cytoplasm frequently contains one or more yolk spheres. The nucleus is at first round in outline and the chromatin is distributed in small nodules lying chiefly against the nuclear membrane. Subsequently it loses its capacity for staining, and appears somewhat like the sperm head, but more rounded. It then begins to increase in size, becoming at the same time irregular in shape and the chromatin once more appears in irregular masses.

As the two pronuclei approach each other their cytoplasmic areas fuse and they come to lie side by side with an amphiaster between (Pl. II., *b*; the aster on the upper side of the nuclei was drawn from the next section). In contact with each pronucleus, may often be seen a large clear vesicle. These probably represent the structures mentioned by Henking as the "descendants of the arrhenoids," *i. e.*, derived from the clear area surrounding the male pronucleus. In Pl. II., *b*, one of the asters contains a minute centrosome. The entire amphiaster is probably formed under the influence of the male pronucleus, for, in the same egg from which Pl. II., *b*, was taken, an accessory sperm nucleus was found with a very small amphiaster lying in contact with it. The further history of the clear vesicles could not be followed as very few first cleavage figures were found; at a later stage of copulation (Pl. II., *c*) they did not appear. Henking

described them in *Pyrrhocoris* as forming the poles of the first cleavage spindle ("Polkörperchen") and apparently considered them to be archoplasmic masses. That he did not see an aster in front of the male pronucleus nor an amphiaster at copulation, in addition to these structures, is perhaps due to his methods of technique.

During the approach of the pronuclei the chromatin in each becomes more and more condensed until the compact somewhat elongated chromosomes appear. Pl. II., *c*, the single example of this stage found, shows the pronuclei of *Archimerus*, still slightly separated. An indistinct aster appears at the right. In the lower nucleus seven chromosomes of different sizes can be distinctly seen. The *m*-chromosome is missing and because of its small size could not be identified in the next section. There are no nucleoli in either pronucleus.[11] The chromosomes in the upper nucleus are not yet fully condensed. The two pronuclei are so nearly equal in volume that one cannot distinguish which is male and which female even before copulation (Pl. II., *c*). It is apparent from a comparison of Pl. II., *b* and *c*, that both undergo a marked decrease in volume just before their nuclear membranes fade out. Pl. II., *d*, shows a late copulation stage or prophase of the first cleavage spindle of *Protenor* in polar view. The nuclear membranes have faded out but the chromosome groups derived from each pronucleus are still separate. This figure is obviously incomplete but it shows distinctly one reduced group (at the right of the figure) in which all the chromosomes appear, seven in number. Just as in the first oöcyte division, the idiochromosome is here recognizable by its relatively large size, and does not in any way resemble a nucleolus. A next largest chromosome and an *m*-chromosome also can be identified, the remaining four being intermediate in size. The group at the left of the figure shows the idiochromosome and three others, the remaining chromosomes being too crowded in the next section to identify. Since each group contains an idiochromosome it is not possible to say which was derived from the egg nucleus and which from a sperm of the class which bear this chromosome.

[11]Stevens ('06*a*, Pl. IV., Fig. 119) has figured this stage in the "Goumi aphid" where there are five chromosomes in each pronucleus, and, in the female, two plasmosomes in addition.

However, the embryo arising from this union would have been a female, for all the products of the first cleavage nucleus would contain two idiochromosomes as in the oögonia. A chromosome group taken from a female embryo is shown in Fig. 12, *c*. Even these meagre results make it probable that the chromosomes coming out of the male and female pronuclei at copulation are of the same number and show the same relative size differences as those which previously entered into the formation of the gametic nuclei.

D. *The Cleavage and Blastoderm Nuclei.*

The cleavage nuclei are formed by successive division of the fertilization nucleus. After each division the daughter nuclei move apart, each surrounded by a star-shaped cytoplasmic island. They wander toward the periphery, continually dividing by mitosis and there form the blastoderm. No instances of amitosis were observed in these stages such as Wheeler ('89) described in *Blatta*. Although but few first cleavage divisions were found, the chromosomes in them do not differ from those of somewhat later stages described beyond. The cleavage mitoses all show spindles, centrosomes and asters with diagrammatic clearness and the chromosomes, though somewhat elongated, can be counted as readily as in the oögonial or spermatogonial divisions. In metaphase each chromosome appears on the spindle split lengthwise and in anaphase the halves separate as in ordinary homotypic division. In telophase the chromosomes at either pole become vesicular, fuse together and form a daughter nucleus. At first the contents of the resting nucleus entirely lacks staining power, no nucleoli of any kind appearing. As the time for the subsequent division approaches, small flakes of chromatin appear which increase in number and gradually unite to form the chromosomes. In the cleavage stages, no definite chromatin nucleoli or plasmosomes could be seen with the methods of fixation employed, nor was there any elimination of chromatin during the earlier mitoses as described by Boveri in *Ascaris*.

1. *Archimems alternatus.*

A careful study of the eggs after fertilization revealed the fact that there are two sorts of embryos, one having 15 chromosomes

in all its cleavage—and blastoderm—nuclei and the other 16. These chromosome numbers are the same as those found in the spermatogonia and oögonia respectively. The size-relations also

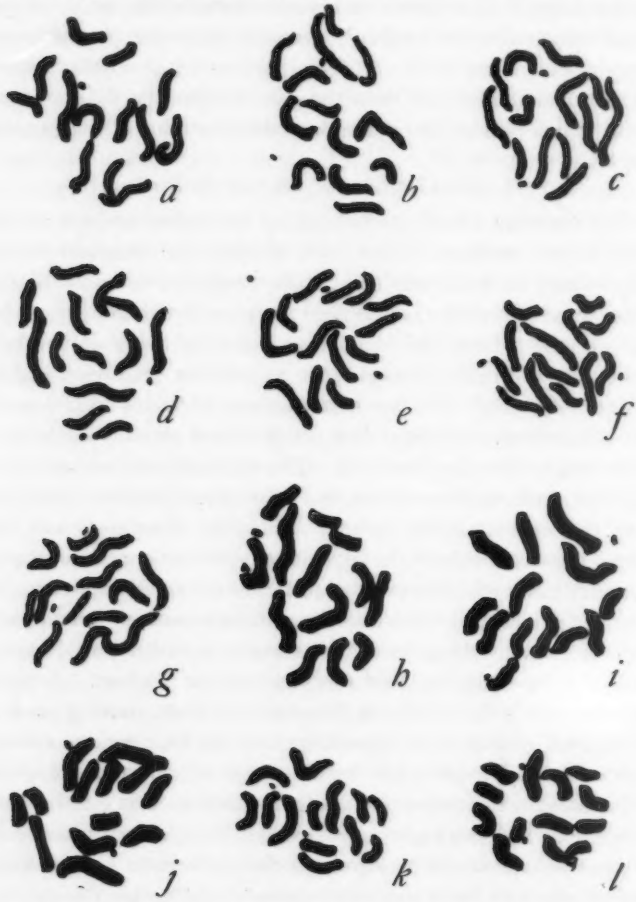

FIG. 6. *Archimerus alternatus*. Chromosome-groups of embryonic cells, 15-chromosome type. *a–e*, from the same embryo; *f–l*, from other embryos.

are in general the same as in the gonads. It seems fair then to conclude that the 15-chromosome embryos are males, the 16-chromosome, females. In Fig. 6 are shown twelve 15-chromo-

some groups taken from embryos at different stages. Fig. 6, *a*, *b*, *c*, *d* and *e*, are from the same embryo early in the formation of the blastoderm. Fig. 6, *f* and *g*, are from another embryo in the same stage. Fig. 6, *h* and *i*, are from an embryo in a slightly later stage of the blastoderm. Fig. 6, *j*, is from still another at the same stage as the last, and *k* from a late blastoderm. Fig. 6,

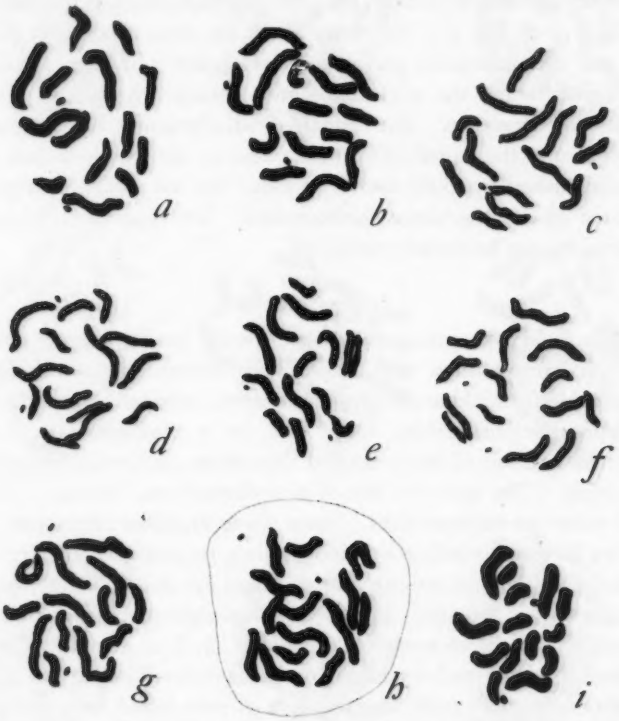

FIG. 7. *Archimerus alternatus*. Chromosome-groups of embryonic cells, 16-chromosome type. *d*, *e*, and *f*, from the same embryo; others from different embryos.

l is taken from a stage in which the blastoderm is invaginating to form the embryonic fundament and membranes. The 16-chromosome groups are shown in Fig. 7. Fig. 7, *a*, shows a group taken from the interior of an egg in the cleavage stage. Fig. 7, *b* and *c*, are from an embryo in the early blastoderm stage.

Fig. 7, *d*, *e* and *f*, are from another embryo in the same stage as the last. Fig. 7, *g* and *h*, are from a slightly later blastoderm and *i* is from a completed blastoderm.

An inspection of Figs. 6 and 7 as a whole, shows that in the earlier stages, the chromosomes are somewhat elongate (Fig. 6, *a–g*; Fig. 7, *a–f*) and that as development proceeds, they become shorter and thicker until at the time of invagination or just before (Fig. 6, *k–l*; Fig. 7, *i*) they have about the same contour as those of the spermatogonia and oögonia (compare with Fig. 1, *a–d*). In every stage the *m*-chromosomes, though very minute, are constant elements. The unpaired idiochrosome in the male groups and the paired idiochromosomes in the female cannot be distinguished by their size or contour but are probably represented among the larger chromosomes. The remaining chromosomes cannot be readily paired off.

2. *Anasa tristis.*

The embryonic mitoses of *Anasa*, though having a larger number of chromosomes that those of *Archimerus*, are much more favorable for making chromosome counts, especially in the early (incomplete) blastodern stage, *i. e.*, at a time when many of the cleavage nuclei have reached the surface and are still rapidly dividing. The embryos are of two classes: one having 21 and the other 22 chromosomes. Since these numbers correspond to those in the spermatogonia and oögonia respectively, it may be concluded that the 21-chromosome class are males, the 22-chromosome class, females. Fig. 8, *a–h*, show eight metaphase groups from the 21-chromosome class. Fig. 8, *a*, *b*, *c*, *d*, *e* and *f*, are taken from an embryo in the early blastoderm stage. In this embryo ten more perfectly clear groups were found each with 21 chromosomes, making sixteen in all from the same embryo. Fig. 8, *g* and *h*, are from another embryo in the same stage. In Fig. 9 six groups of the 22-chromosome class are shown, all from the same embryo in the early blastoderm stage.

One exceptional group was found in an embryo of the 22-chromosome class (Fig. 8, *i*). This group contains 23 chromosomes, of which three are larger than the rest. It is difficult to suggest an explanation for this condition. It may be due to an

accident of technique, the microtome knife tearing a chromosome in half as it passed through the block, or it may be the result of an abnormality in a previous division. There were no other

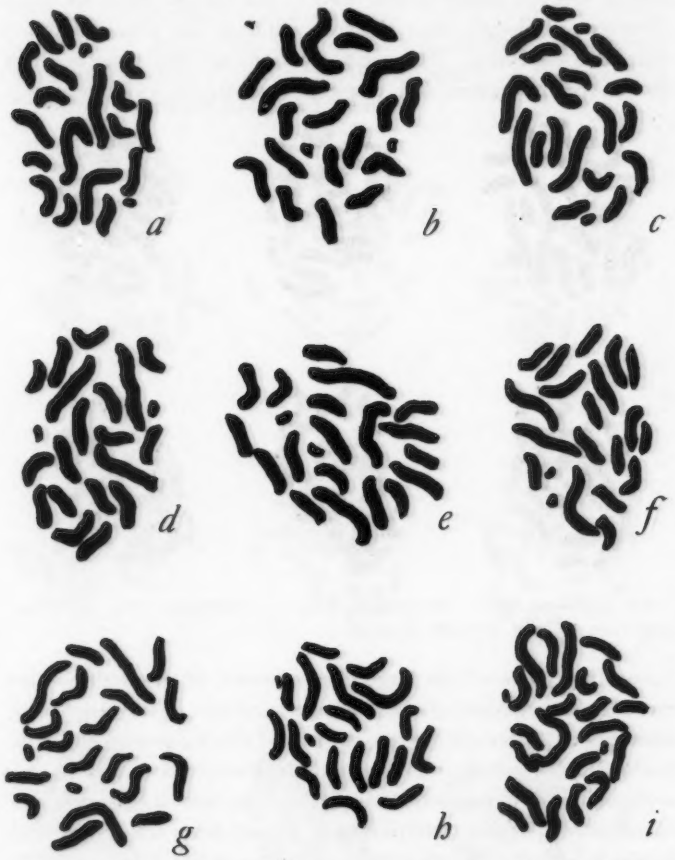

FIG. 8. *Anasa tristis*. Chromosome-groups of embryonic cells. *a–h*, 21-chromosome type: *a*, *b*, *c*, *d*, *e* and *f*, from the same embryo; *g* and *h*, from another embryo. *i*, exceptional group with twenty-three chromosomes.

mitoses in the immediate vicinity from which an extra chromosome could have been derived. The chromosome groups represented in Figs. 8 and 9 were selected from a large number of

very clear preparations. Many more could have been shown, but it seemed needless to multiply the number of figures.[12]

A comparison of the male groups (Fig. 8, *a–h*) with the female groups (Fig. 9) shows clearly that in the former there are three chromosomes larger than the rest, while in the latter there are four such elements. These size relations are the same as those in the spermatogonia and oögonia respectively (vid. page 89).

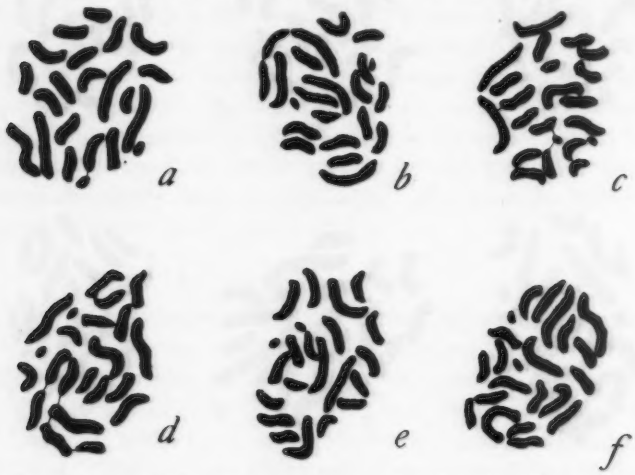

FIG. 9. *Anasa tristis*. Chromosome-groups of embryonic cells, 22-chromosome type, all from the same embryo.

Accordingly, one of the large chromosomes of the male groups must be the unpaired idiochromosome and two of the large chromosomes of the female groups, the paired idiochromosomes. The *m*-chromosomes appear as constant elements in both groups and are usually more elongated than in the germ-cells, a feature which is common to all the chromosomes. Apart from the largest and smallest elements, this elongated condition makes it impossible to pair off the remaining chromosomes with any degree of certainty.

[12]All the figures were drawn with camera lucida, Zeiss apochromat. 2 mm., compens. oc. 12. With the exception of Plates I. and II., they were again enlarged with the camera and subsequently reduced in reproduction one-half, giving a final magnification of 2,650 diameters. The magnification of Plates I. and II., is 1,375 diameters. Achromatic structures, except those of Plates I. and II., have been represented semi-schematically.

3. *Chelinidea vittigera.*

The embryonic groups of this species are very similar to those of *Anasa* and equally favorable for making chromosome counts. The embryos are again of two sorts, one having 21 chromosomes, the other 22. In fact the number and size-relations are so much like those in *Anasa* that the two forms cannot be distinguished by their chromosome complexes alone.

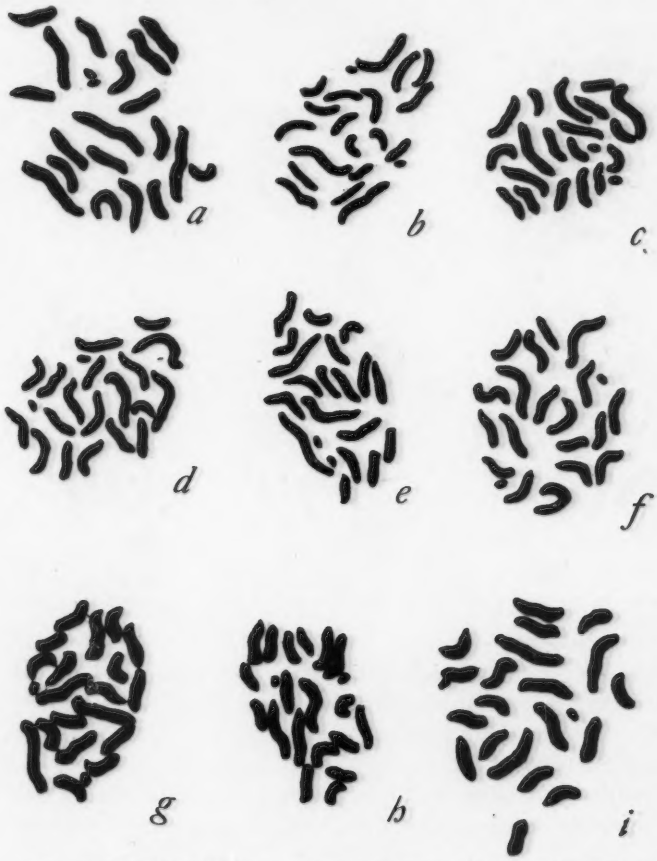

FIG. 10. *Chelinidea vittigera.* Chromosome-groups of embryonic cells. *a–h*, 21-chromosome type; *b*, *c*, *d*, *e* and *f*, from the same embryo; *a*, from an early cleavage. *i* exceptional group with twenty-two chromosomes.

In Fig. 10, *a–h*, are shown eight 21-chromosome groups. Fig. 10, *a*, is from an early cleavage stage corresponding approximately to the fourth cleavage of holoblastic eggs.[13] Fig. 10, *b*, *c*, *d*, *e*, and *f* are from an early blastoderm stage. Seven more perfectly clear counts were made in this embryo, all giving 21 chromosomes, making twelve in all. Fig. 10, *g* and *h*, are from another embryo in the same stage. Fig. 11 shows six of the 22-chromosome groups. Fig. 11, *a*, is taken from an early cleavage

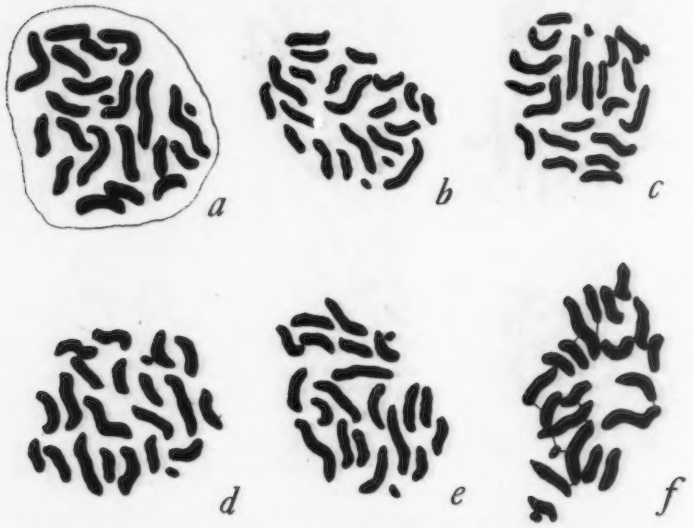

FIG. 11. *Chelinidea vittigera*. Chromosome-groups of embryonic cells, 22-chromosome type. *b*, *c*, *d* and *e*, from the same embryo; *f*, from another embryo; *a*, from an early cleavage.

stage, approximately the fourth. Fig. 11, *b*, *c*, *d* and *e*, are from an early blastoderm stage in which eleven perfectly clear groups were found all with 22 chromosomes. Fig. 11, *f*, is from another early blastoderm.

As in *Anasa*, it seems fair to conclude that the embryos with 21 chromosomes are males, those with 22 chromosomes, females,

[13]After the "second cleavage," the nuclei apparently do not divide quite synchronously so that one may find at times an odd number of nuclei, some resting and some in division.

for these groups correspond in number and size-relations with those of the spermatogonia and oögonia respectively. Like *Anasa* again, the male groups contain three chromosomes which are distinctly larger than the others (Fig. 10, *a–f* and *h*), one of them probably representing the unpaired idiochromosome. In the female groups four largest chromosomes can frequently be distinguished (Fig. 11, *b*, *c* and *f*), though they cannot be identified in all the figures, probably on account of fore-shortening. Two of these may be considered as the paired idiochromosomes, in place of the unpaired element of the male groups. The *m*-chromosomes are typically paired elements of both male and female groups. All the chromosomes as in *Anasa* are more elongated than in the gonads but still preserve the same size-relations. The groups shown in Figs. 10 and 11 were selected from a large number of clear preparations. Fig. 10, *i*, is a group of 22 chromosomes taken from an embryo in which all other counts gave 21. For this, the single exception of its kind observed, it is difficult to give a satisfactory explanation, though the same possibilities suggested in connection with the exception found in *Anasa* (vid. page 102) might also apply here. There were no neighboring groups from which the extra chromosome could have been derived.

4. *Protenor belfragei.*

The embryonic mitoses of this species are not quite so favorable for making chromosome counts as those of the two preceding forms, on account of the elongation of the chromosomes in the early and even late cleavage stages. In the blastoderm stage the chromosomes are more compact, but very few embryos were obtained at this time so that the results are very meagre. Fig. 12 shows two 13-chromosome groups (*a* and *b*) taken from an early blastoderm stage, and one 14-chromosome group (*c*) from another embryo in the same stage.

In the 13-chromosome groups (Fig. 12, *a* and *b*) a very large chromosome, unquestionably the unpaired idiochromosome, stands out clearly, being more than twice as large as any other. A second largest pair can also be readily identified and a smallest pair, the *m*-chromosomes.

In the 14-chromosome group (Fig. 12, *c*) there are two very large chromosomes equal in size, in place of the unpaired element of the first two groups. These are no doubt the paired idiochromosomes. There is also a next largest pair but the *m*-chromosomes cannot be identified with certainty.

A comparison of these embryonic mitoses (Fig. 12) with those of the gonads (Fig. 5, *a–d*) shows that the 13-chromosome groups are similar in the number and size-relations of their chromosomes

FIG. 12. *Protenor belfragei*. Chromosome-groups of embryonic cells. *a* and *b*, 13-chromosome type, from the same embryo. *c*, 14-chromosome type, from another embryo.

to those of the spermatogonia, the 14-chromosome group, to those of the oögonia. Though the results are too few to justify broad conclusions, it is most probable that the embryo with 13 chromosomes is a male, the one with 14 chromosomes a female, thus bringing *Protenor* in line with *Archimerus*, *Anasa* and *Chelinidea*.

IV. SUMMARY AND CONCLUSION.

Among the results described in this paper,[14] those of particular interest are as follows:

1. In *Archimerus*, *Anasa* and *Protenor* there is an odd or unpaired chromosome in the spermatogonia which in *Protenor* is distinguishable by its size. The oögonia contain in addition to this chromosome, a second chromosome of the same size. These observations are in agreement with those of Wilson, Montgomery and of Lefevre and McGill for the forms mentioned.

2. The chromosomes in the reduced female groups (polar or oöcyte divisions) show the same relative size differences as the corresponding pairs in the oögonia (particularly well shown in *Protenor*).

3. All the chromosomes divide in both polar divisions (proof

[14]A preliminary note giving the most important results was published in *Science* for December 31, 1909.

decisive in *Archimerus*, less complete in *Anasa* and *Protenor*). There are no peculiar or "lagging" chromosomes in either of these divisions.

4. The female pronucleus contains a group of chromosomes similar to that borne by a spermatozoön having the "accessory" or idiochromosome (directly proved in *Archimerus*).

5. At fertilization the reduced groups from each pronucleus are separately distinguishable and the chromosomes show the same size relations as those of the spermatocyte and oöcyte divisions. There are no nucleoli in either pronucleus.

6. In the cleavage and early blastoderm nuclei of *Archimerus*, *Anasa*, *Chelinidea* and *Protenor*, the chromosomes are perfectly distinct and can be counted as readily as those in the gonads. Two types of embryos are found, one having an odd and the other an even number of chromosomes, these numbers being respectively the same as occur in the spermatogonia and oögonia. Accordingly it seems fair to conclude that the former are males, the latter females, and it thus becomes possible to distinguish the sex of an embryo by counting its chromosomes.

7. The idiochromosomes behave exactly like the other chromosomes, in the oöcyte divisions, at fertilization and in the cleavage and early blastoderm stages. They never show any resemblance to nucleoli and in *Protenor* they can be identified in all stages with absolute certainty.

It will be seen that the results in general bear out the assumptions made by Stevens, Wilson and others regarding the number and behavior of the chromosomes in the maturation of the female and in the somatic cells. They give additional morphological support to theories of sex-production based upon the presence or absence of certain chromosomes and to the hypothesis of chromosome-individuality or "genetic continuity of chromosomes" as Wilson ('09*c*) more cautiously calls it.

V. Review and Discussion.

The literature on the maturation and early development of the eggs of insects and allied forms is very extensive, covering a period of over fifty years, but it is beyond the scope of the present paper to review it in detail except in so far as it concerns

the history of the chromatin in the early stages. Considered from this standpoint, the results briefly are as follows:

Diptera.—Apart from the earlier works of Weismann and Blochmann in which the chromosomes were not especially considered, there are no observations except those of Henking ('88 and '93) on *Musca vomitoria*. In the first paper Henking figured the cleavage spindles but did not determine the number of chromosomes. Moreover, the results are difficult to interpret because of the standpoint taken in regard to "free-nuclei-formation." In the later paper Henking summarizes his previous results but gives no new observations.

Lepidoptera.—Platner ('88) described briefly the maturation and early cleavage of the parthenogenetic and fertilized eggs of *Liparis dispar* but gave no figures and no account of the chromosomes. Henking ('90) described and figured the maturation, fertilization and early cleavage of *Pieris brassicæ*. He found the haploid number of chromosomes to be 14 in both polar spindles and in the female pronucleus, but did not accurately determine the diploid number, though in a later paper he gives the probable number as 28. The same author ('92) gave a brief account of the maturation and early cleavage of *Bombyx mori* and *Leucoma salicis* in which the haploid chromosome-group was stated to be "at least 12," in both species. The diploid number was not determined.

Neuroptera.—As far as I am aware, there are no observations on the maturation and cleavage of mitoses of the eggs of this group. Miss McGill's ('06) observations on *Anax junius* and *Plathemis lydia* were confined to the nuclear changes during the growth period of the oöcytes.

Coleoptera.—Wheeler ('89) observed the formation of the first polar spindle of *Leptinotarsa* (*Doryphora*) *decemlineata* but did not determine the number of chromosomes. Henking ('92) in *Agelastica alni* found the haploid number of chromosomes to be about 12 in both polar spindles and the diploid number, 24–30 in the cleavage spindles. He also observed the approximate number in *Lampyris splendidula*, *Adimonia tanaceti*, and *Donacia* (*sericea* L.?). In none of these, however, did he observe the diploid groups in the cleavage stages. The observations of

Giardina ('01) on *Dytiscus marginalis* and likewise those of Debaisieux ('09) on the same species were confined to the growth period of the oöcytes. Both authors describe a large chromatic mass in the nucleus of the oöcyte, distinct from the chromosomes, which appears to be eliminated just before the maturation divisions. The latter are not described.

Orthoptera.—The observations of Blochmann ('87) and Wheeler ('89) on *Blatta germanica* were not very extensive from our point of view. Neither author determined the number of chromosomes in the maturation spindles. Wheeler, however, gives a good figure of a cleavage spindle showing 10 chromosomes. Gutherz ('07) in a brief paper, described a chromosome-nucleolus in the oöcytes of *Pyrrhocoris* but found no such body in the somatic mitoses of *Gryllus domesticus*. He therefore questioned the occurrence of "heterochromosomes," maintaining that there were probably 20 chromosomes in the somatic cells of the last named species and no "heterochromosomes." However in his later papers ('08 and '09*a*) he abandoned this view, describing typical "heterochromosomes" in the spermatogonia and oögonia of *Gryllus* as in other Orthoptera, and stating further, that the somatic cells have the same number of chromosomes as the oögonia and spermatogonia respectively though he gave no observations in support of this last statement. The observations of von Baehr ('07) on the parthenogenetic egg of the phasmid, *Bacillus rossii*, though detailed in some respects, are not quite conclusive in regard to the number of chromosomes. The egg nucleus, just before the first polar division, contains 18–20 chromosomes many of which are tetrads. In anaphase the double nature of the daughter halves often becomes apparent. Moreover, there is one large tetrad in the first division which again appears in the second. The number of chromosomes in the latter division was not determined. In a recent paper Buchner ('09) has described in *Gryllus campestris* an irregular nucleolus-like structure, the "accessory body," which persists through the growth period of the oöcytes. It is derived from a similar body in the oögonia which he apparently considers identical with the "accessory" chromosome of other forms. . The maturation mitoses were not observed. Gutherz ('09*b*) working on a nearly related species, *Gryllus domes-*

ticus, finds a similar body in the growth period of the oöcytes. After tracing its history, he concludes however that it is comparable to a nucleolus and is not to be confused with a "heterochromosome." Buchner's identification of the "accessory body" as a chromosome thus appears very doubtful.

Hymenoptera.—Blochmann's ('89) observations on the maturation of the fertilized and parthenogenetic egg of the bee were concerned chiefly with the number of polar bodies formed in the two sorts of eggs. Though the polar spindles were figured the exact number of chromosomes was not determined. Henking ('92) found the haploid number of chromosomes to be 10 in the polar spindles of *Lasius niger* and the diploid number in the cleavage spindles, 20. In the unfertilized egg of *Rhodites rosæ* he found 9 chromosomes in the polar spindles. In the cleavage nuclei the number was 18–20, *i. e.*, the number of chromosomes in the female pronucleus had been doubled. Petrunkewitsch ('01) working on the fertilized and parthenogenetic eggs of the bee, found that the first polar division was equational, the number of chromosomes being 16. In the second division there occurred in both sorts of eggs, a reduction of the chromosome-number to about half, *i. e.*, from 16 to 8. In the parthenogenetic (drone-) egg, the female pronucleus contained at first 8, but later 16 chromosomes, the latter being produced by a doubling of the haploid group, so that in the equatorial plate of the first cleavage spindle the diploid number, 16, again appeared. In later cleavages there was a progressive doubling of the chromosome-number, producing multiple groups of 32 and 64. Silvestri ('06 and '08) has described in detail the maturation, fertilization and cleavage of several species of parasitic hymenoptera (*Litomastix*, *Encyrtus*, *Oöphthora*, *Ageniaspis*). However, since his results do not include the determination of the exact number of chromosomes in the early stages, it will be unnecessary to review them here. Doncaster's ('07) results on *Nematus ribesii* (Tenthredinidæ) are quite anomalous and difficult to interpret. He finds that there are two types of maturation in the female. In some eggs there is no reduction of chromosomes, the female pronucleus receiving the diploid number, 8. In others typical reduction occurs, the egg nucleus receiving in all probability the haploid number, 4.

The former type of egg develops parthenogenetically, the latter only being capable of fertilization, it is supposed. In some somatic tissues, such as the ovary sheath, there are more than the diploid number of chromosomes, as in the bee and in *Ascaris*. In a later brief communication, however, Doncaster ('10*a*) states that his observations on the polar mitoses may require revision and that the behavior of the chromosomes in *Nematus ribesii* is so difficult to follow that it is doubtful if a satisfactory interpretation can be obtained in this species. In a very recent paper, the same author ('10*b*) describes in detail the maturation, fertilization and early cleavage of *Neuroterus lenticularis* (Cynipidæ). Here again some of the results are quite novel. The mitoses of the primitive ova found in young female larvæ of the summer generation contain about 20 chromosomes, like those of the somatic cells. In the maturation of the summer eggs apparently two divisions occur, the female pronucleus probably containing 10 chromosomes. The eggs are fertilized and in the cleavage spindles about 20 chromosomes appear. The results on the maturation of the spring (parthenogenetic) egg are so anomalous that it seems best to quote from Doncaster's own summary (*loc. cit.*, p. 102): "The maturation of the spring egg has not yet been sufficiently studied, but it appears that some eggs undergo at least one maturation division, others probably none. In eggs in which maturation has occurred segmentation mitoses show 10 chromosomes; all the eggs laid by one individual female in which the chromosomes could be counted were of this type, and it is suggested that these develop into males. In the eggs laid by other females, however, 20 chromosomes appear in the segmentation divisions; in these, polar chromosomes appear to be absent, and it is probable that there has been no maturation division, and that these eggs would develop into females." It will be seen that no definite conclusions can be drawn without further confirmatory observations. Schleip's ('08) observations on the polar body formation in *Formica sanguinea* were confined chiefly to the parthenogenetic egg. He found in the latter the haploid number of chromosomes to be about 24 in the maturation spindles and female pronucleus. This number also appears in the first cleavage nucleus. In the fertilized egg, the number of chromosomes

in the male and female pronuclei was not determined with certainty but is probably 24 in each. On the whole, the chromosomes were small and the size-differences not well marked.

Hemiptera-homoptera.—The older papers of Weismann, Witlaczil, Blochmann and Will on the early embryology of aphids contain no detailed account of the chromosomes. Stschelkanovzew ('04) in a brief paper on the maturation and early cleavage of the summer (parthenogenetic) egg of *Aphis rosæ*, gave 14 as the number of chromosomes in the maturation spindle (only one polar body is formed). In one first cleavage spindle there were only 11 chromosomes but he considered that three of these might be double elements, thus giving the diploid number, 14, in both maturation and cleavage. Miss Stevens ('05*a*) and Hewitt ('06),[15] however, found the diploid number in the parthenogenetic egg of *Aphis rosæ* to be 10 and this count has been confirmed by von Baehr ('09) in the same species. Miss Stevens also found that the winter (fertilized) egg gave off two polar bodies in which the haploid number, 5, was present. Three of these authors observed marked size differences in the chromosomes of the maturation and early cleavage stages, both Miss Stevens and von Baehr finding four smallest chromosomes constantly. Miss Stevens ('06*b*) in a very extensive paper described the maturation and cleavage in a large number of aphids, with especial reference to the number and behavior of the chromosomes. Without giving a detailed review of her results it may be said in general that the number and size differences of the chromosomes was found to be constant for the species and that this constancy applies to the diploid groups whether in the maturation spindle of a parthenogenetic egg or in its cleavage spindles. In many cases also the haploid group was found to exhibit the same relative size differences as the diploid group of the same species. In the fertilized egg of the "Goumi aphid," the number of chromosomes in the male and female pronuclei just before copulation was shown to be 5 in each, *i. e.*, the haploid number. Miss Stevens concluded from her observations on aphids up to this time that there were no "heterochromosomes" in this group, but

[15]Hewitt's results are known to me only through the brief mention made by von Baehr ('09, p. 285).

more recently ('09) she has abandoned this view and has reached conclusions in agreement with those of Morgan and von Baehr, mentioned below. The results of Tannreuther ('07) on the maturation and cleavage of several species of aphids differ in many important respects from those of other workers on the same group. They have been questioned by Morgan ('09) and von Baehr ('09) and will not be considered here. Morgan ('08 and '09) has traced the full history of the chromosomes through several generations of phylloxerans. He observed that the parthenogenetic eggs of the second generation, *i. e.*, those which produce the sexual individuals, are of two sorts both as to their size and the number of chromosomes in the embryos which they produce. The male embryos have actually two less chromosomes than the female though this difference is not always apparent owing to fusions occurring between certain chromosomes (the "accessories.") The embryonic chromosome-groups of female individuals contain four "accessory" chromosomes. Those of the males, however, have but two "accessories" since although males are produced parthenogenetically from females, two of these chromosomes are given off to the polar body in the maturation of the male-producing egg. The most important conclusions to be drawn from these results for our purposes are, that idiochromosomes are present throughout the entire life-cycle and that it is possible to diagnose the sex of an embryo by counting its chromosomes, though here it is true, sex is also associated with the size of the egg. Von Baehr ('09) described the maturation and cleavage of the parthenogenetic eggs of several species of aphids. His results were in general similar to those of Miss Stevens, the maturation and cleavage mitoses being similar in the number and size relations of the chromosomes. He did not observe any elimination of "accessory" chromosomes in the polar division of male eggs as in the phylloxerans. However, in one maturation spindle of *Aphis saliceti* (*loc. cit.*, Pl. XIV., Fig. 42) he figures 5 chromosomes, the remaining figures showing 6, and in a male somatic cell (Pl. XV., Fig. 94), as well as in the spermatogonia, 5 chromosomes again appear. Moreover his results on the spermatogenesis of *Aphis saliceti* as well as those of Miss Stevens ('09) on the spermatogenesis of other aphids would seem

to indicate that the behavior of the chromosomes in the female line is probably similar to that in phylloxerans.

Hemiptera-heteroptera.—The only observations on the maturation and cleavage of the egg in this group are those of Henking ('92) on *Pyrrhocoris*. He has given a very extensive and detailed account of the chromosome history in this form and in a previous paper ('91) described the spermatogenesis. He found that in the diploid groups of the oögonia, there were 24 chromosomes. The follicle and connective tissue cells, both larval and adult also showed this number. In the haploid group of the first polar spindle 12 dumbbell-shaped chromosomes appeared. In one such group ('92, Pl. III., Fig. 83) one chromosome is much larger than the rest and is probably the idiochromosome pair (cf. Wilson's ('09*d*) figures of the oögonial groups). The second polar spindle showed again 12 dumbbell-shaped chromosomes. The number of chromosomes in the male and female pronuclei was not accurately determined but in Henking's Fig. 90 (Pl. III.) one such nucleus shows 12 chromosomes. The early cleavage spindles were figured but of them the author says (*loc. cit.*, pp. 29–30): "The number (of chromosomes) cannot be accurately determined on account of the smallness of the spindle and the close grouping of the chromosomes . . . it should be 24." He thus did not distinguish two classes of embryos with reference to the chromosome number. This, no doubt, was partly due to the fact that he had not observed any difference in the number of chromosomes in the spermatogonia and oögonia and did not appreciate the significance of the idiochromosome ("accessory" chromosome) which he himself was the first to describe. Foot and Strobell ('09) have described the growth period of the oöcytes of *Euschistus variolarius*. In accordance with the earlier account of Wilson ('06), they find no chromatin nucleolus in the young oöcytes or germinal vesicles of this species but in the older oöcytes and in the germinal vesicles there is a relatively large achromatic nucleolus. The maturation divisions were not described.

Arachnida.—Montgomery's ('07) results on *Theridium* are not very extensive from the chromosome-standpoint. He found in the second polar spindle 12 chromosomes and in a fourth cleavage spindle 24 chromosomes. No idiochromosomes were observed.

Excluding the early cleavage groups mentioned above, a number of authors have described the chromosomes of older somatic cells. Henking ('92) found the number of chromosomes in the egg-follicle and connective tissue cells, of *Pyrrhocoris* to be 24. Petrunkewitsch ('01) observed that young blastoderm-cells of the bee contain multiple groups. Miss Stevens ('05*b* and '06*b*) described the somatic groups of several species of Coleoptera and found that the small idiochromosome which occurs only in the male of these forms could be readily identified. Von Baehr ('09) observed that the male somatic groups of *Aphis saliceti* contain 5 chromosomes, one less than the female. Gutherz ('09*a*) concluded that in *Gryllus domesticus* the somatic cells have the same number of chromosomes as the oögonia and spermatogonia respectively, though his observations on this point were not very extensive and no figures of somatic mitoses were given. Doncaster ('10) observed the somatic groups in the male and female pupæ of the gall-fly. He found that in the male, some somatic mitoses show the diploid number of chromosomes while others may show the haploid number. In the female, all somatic mitoses have the diploid number. The very anomalous conditions described for the male do not at present rest upon demonstrative evidence as the chromosomes were found to be small and difficult to count. In addition to the above-mentioned observations, most recent papers on the spermatogenesis of insects contain accounts of the oögonial groups in which idiochromosomes can often be identified.

From the foregoing brief view of the literature on the chromosomes in oögenesis and cleavage, it is evident that with the exception of Miss Stevens and Morgan none of the authors have traced the idiochromosomes into the cleavage and later somatic mitoses, and none but Miss Stevens, Morgan and von Baehr have shown that the embryonic or larval somatic cells of male individuals differ from those of females in the number or size of their chromosomes. Morgan has also shown that idiochromosomes are present in the polar spindle where, in his material, they behave in a characteristic manner. The results on the whole show, I think, that idiochromosomes ("heterochromosomes") are constant chromosome-elements and not merely temporary structures (nucleoli) present during maturation.

Outside of the air-breathing arthropods, there are, as mentioned before, two other groups in which idiochromosomes or similar structures have been found in maturation and cleavage. Baltzer ('08) has found that in two species of sea-urchins there is a particular hook-shaped chromosome which occurs in only a part of the mature eggs. The eggs are thus of two types with respect to this element. (It is replaced by a chromosome of the ordinary sort in the eggs which lack it.) The sperm nuclei on the contrary are all alike. It is not improbable, Baltzer concludes, that the determination of sex depends upon this dissimilarity of egg nuclei, and therefore *lies with the female* (*i. e.*, with the egg), as in the male and female (parthenogenetic) eggs of aphids and phylloxerans. The peculiar hook-shaped elements might thus be called "idiochromosomes." Eggs which contain this element would develop into females, those without, into males. In a very recent paper Boveri and Gulick ('09) have described briefly the chromosome-cycle in *Heterakis*, a nematode. Its cycle corresponds exactly with that of *Protenor* as given by Wilson ('06). The diploid number in the male (spermatogonia) is 9. During spermatogenesis the odd chromosome goes undivided to one pole of the spindle in the first spermatocyte division but divides in the second. The spermatozoa are thus of two classes, with 5 and 4 chromosomes respectively. The diploid number in the female was not determined with certainty but the haploid number in the germinal vesicles and polar spindles was found to be 5. The eggs are thus all alike and, it is assumed, will develop into males or females according as they are fertilized by 4-chromosome or 5-chromosome spermatozoa. The chromosomes of the cleavage nuclei were not described.

Since the results here described for coreid Hemiptera do not give any further insight into the fundamental question of sex-determination but only render the data more complete, it seems needless to add a lengthy discussion on this point. In the recent papers of Wilson, Bateson Castle, Boveri and Morgan, the cytological evidence relating to sex-determination has been thoroughly analyzed. It may be pointed out, however, that apart from theoretical considerations this evidence has been questioned from the standpoint of fact by several workers who have supported their

contentions by direct observations on insect spermatogenesis. Arnold ('08) from his observations on the spermatogenesis of *Hydrophilus piceus* concluded that there were no idiochromosomes in that form, although Miss Stevens has found these elements in all the Coleoptera which she has examined (42 species). The objection offered by Foot and Strobell ('07) to the presence of an odd number of chromosomes in the spermatogonia of *Anasa tristis*, hence of an "accessory" chromosome and the replies to this objection have already been considered. The present work, particularly the section dealing with the cleavage and early blastoderm nuclei, gives further proof that in this species, as well as in the other three examined, the number of somatic chromosomes in the male is one less than in the female. Gross, from his studies on *Syromastes marginatus* ('04) and *Pyrrhocoris apterus* ('06), concluded that the "accessory" chromosome could have no effect on sex-production in these two forms, for he believed that the number of chromosomes is the same in both sexes—22 in *Syromastes* and 24 in *Pyrrhocoris*. In the last named form his counts agree with the earlier ones of Henking ('91), though the latter was uncertain of the spermatogonial number. Recently however Wilson ('09*b*, '09*d*) has reëxamined both these forms and finds that in *Pyrrhocoris*, the male has one less chromosome than the female, *i. e.*, 23 instead of 24, while in *Sryomastes*, the male has 22 as described by Gross but the female has 24 instead of 22. *Pyrrhocoris* may thus be plaecd in the same class with such forms as *Archimerus*, *Anasa* and *Protenor*. *Syromastes*, however, is unique among the Hemiptera heteroptera in having a bivalent "accessory," though a similar condition has been described by Morgan ('09) in the homopteran, *Phylloxera caryæcaulis*, while Payne ('09) describes several cases among the Reduviidæ (*Fitchia*, *Rocconota*, *Conorhinus*) in which the large idiochromosome (which represents the "accessory") is double.

In addition to the objections cited above, a number of authors have either expressed their doubts of the presence of two sorts of spermatozoa, or, while admitting the existence of such a dimorphism, have questioned its sexual significance. In recent years, however, evidence has been steadily accumulating in support of the conclusion that a nuclear dimorphism of the sperm—

or of the eggs—does occur, not only in insects but in at least two other groups of animals and that it bears a definite relation to sex-production. Whether the sexual tendencies are carried by specific chromosomes or whether certain combinations of chromosomes cause one sex or the other to arise, is still an open question, but that it is possible to demonstrate a nuclear difference in the gametes, and frequently indeed, in the somatic tissues of the two sexes, is now, I think, placed beyond doubt by many decisive observations.

April 27, 1910.

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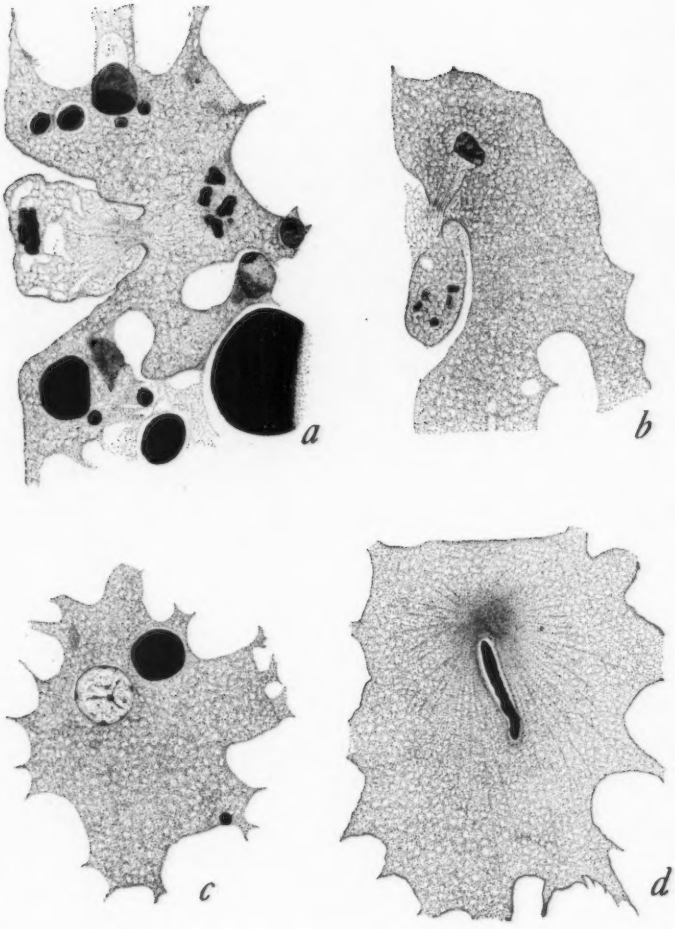
Explanation of Plate I.

Archimerus alternatus. *a*, formation of the first polar body—the mitotic figure is in final anaphase, the inner daughter group showing eight chromosomes; the cytoplasm contains a number of yolk spheres of different sizes and staining capacity. *b*, formation of the second polar body—the inner group of chromosomes have been transformed into the female pronucleus; those of the outer group are still separate. *c*, the female pronucleus advancing into the egg in its cytoplasmic "island"; the latter also contains two yolk spheres. *d*, the sperm-head advancing into the egg, in its cytoplasmic "island"; it is preceded by an aster and surrounded by a clear area. The magnification is 1,375 diameters.


a
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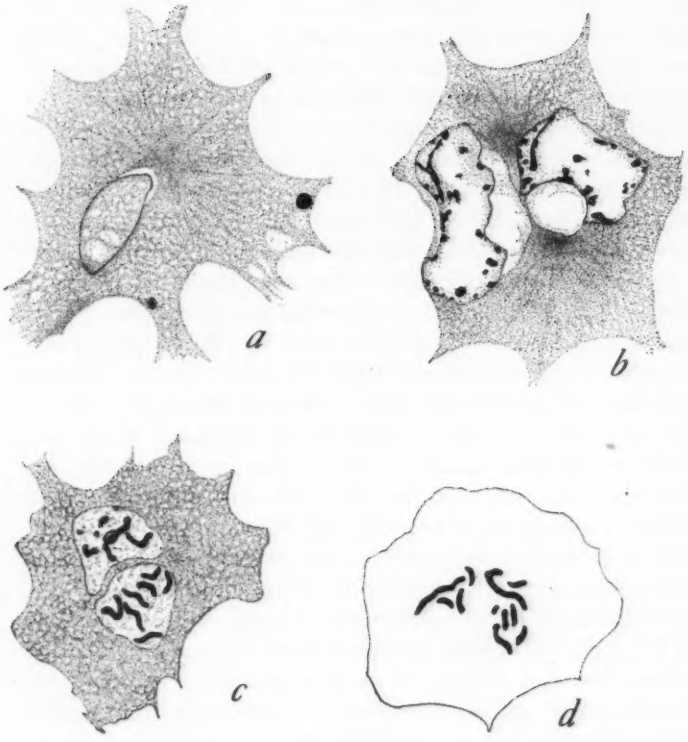
Explanation of Plate II.

a–c, Archimerus alternatus. *a*, a stage letter than Pl. I., *d*—the sperm head has been transformed into the male pronucleus and is still advancing into the egg preceded by its aster and a small clear area. *b*, an early stage in the copulation of the male and female pronuclei—each is in contact with a clear area and an amphiaster lies between them. *c*, a later stage in the copulation of the pronuclei—the nuclear membranes are still intact; seven chromosomes appear in the lower nucleus, the *m*-chromosome being absent; a faint aster appears on the right. *d, Protenor belfragei:* First cleavage prophase, polar view—the male and female groups are still separate, one of them being incomplete; an idiochromosome appears in each group. The magnification is 1,375 diameters.


a
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COURTSHIP IN DYSDERA CROCATA.

ALEXANDER PETRUNKEVITCH, PH.D.

A female and a male of this species of spiders were found under two adjacent flat stones on a sunny hillside in Montclair on the morning of April 21. On the same day they were put in a rectangular small glass dish with a little earth at the bottom, a wire-net lid and a partition of wire-net in the middle to separate the spiders from each other. They at once tried to get at each other, sticking their legs through the net. After a while they desisted and sat quietly on the ground. The next morning the female was found in the corner farthest from the light and next to the screen partition, in a little excavation in the ground, which was protected on all sides with web. The web was destroyed and a fly was held in a forceps before the spider. She bit at it but was either unable or did not want to kill it. The male made no excavation or web. When the partition was removed the male and female met several times in a rather threatening way, opening wide their mandibles and at times seizing each other by the mandibles, but the male would pat the female with his four front feet on the sides and back and then they would separate peaceably. After a short time the female dug rapidly a hole in the ground under a little stone. The male approached several times, touching her legs with his front legs and again going away. I now removed the stone to observe how the female digs the hole. She at once began to work, using all parts of her body. The small clumps of soil she removes by pushing them with palpi, front feet and mandibles with the fangs drawn in. Heavy clumps of the size of her own body she grasps in her fangs and either pushes or pulls backward out of the excavation. The hole is fortified by web which she spins in a very interesting manner. She stands head down with only the abdomen out of the hole and moves the latter in a half circle about the petiolus with spinnerets outstretched, fastening the thread first at one, then at the other end of the half circle. After doing this several times, she turns in the hole to spin the other half circle in the same manner.

Meanwhile the male came quite close and was evidently watching her, at the same time cleaning his legs and palpi by drawing them through his mandibles. After a while he again began his courtship; patted her back, standing face to face and tried to bring his front legs under her body. Later she left the hole without finishing it, returning to it from time to time. They met several times on the ground, always with mandibles wide open and touching each other with the four front legs. At times the female would make a threatening move toward the male upon which he would draw back, but invariably a rapid trembling would seize his four front legs, which lasted from 10–15 seconds. At times when she was out of the hole as though in search of something, he would occuply her place in it. On returning, she gently displaced him. Later as the female showed no intention of accepting the male, I separated them by means of the wire screen.

The next day was cold and rainy and both spiders were found in the morning in separate excavations covered up with earth and web. On the third day after a stormy night it became warm and the glass was placed in the sunshine. The screen was removed and the spiders pushed out of their holes. The male at once went to the female, meeting her face to face. He crept under her sternum and took her firmly by the petiolus between his mandibles with fangs drawn in. With his legs he embraced her in such a way that his right front leg passed between the mandibles (with fangs open) of the female and rested on her back. His left front leg passed between her right mandible and palpus and also rested on her back. The other legs passed similarly between the legs of the female and touched her back. She seemed to make no objection whatever. They tried several times to change their position, the female creeping from place to place while the male continued to cling to her. Finally she lay down on her side and he applied the palpus (at 10.43 A. M.), all the while patting her with his third leg. The coitus lasted five minutes, after which they slowly separated and going to opposite sides of the dish hid themselves in the ground. During the whole time the back of the male was in contact with the sternum of the female, a position not common to the majority of spiders.

In a recent paper on the courtship of araneids Professor Mont-

gomery objects to my conclusion that the sense of sight is the only sense that guides hunting spiders in finding the females during the mating period. He says that he has "frequently noticed males of even diurnal attids and lycosids first recognizing the female by touch." It seems to me however very doubtful that a male should approach a female without having previously noticed her or without having been noticed by her, so to say unawares on the part of both. The fact that the female may stay quiet is no proof that she has not noticed him. From my observations and experiments on hunting spiders I am convinced that they readily see moving objects. If frightened they run away; if conscious of their advantage and hungry or if cornered, they attack. What Montgomery looks upon as recognition by touch may be merely an attempt on the part of the male to find out whether the female is inclined to accept him. I do not deny that such a chance meeting is possible, but from what we know of the habits of spiders, I should expect the female to be either startled or resentful on being touched unawares. In the case of *Dysdera crocata*, which may in a certain sense be regarded as a hunting spider, the male shows every indication of perceiving moving objects and of recognizing the female by sight. Several times he distinctly watched the end of a hatpin with which I was breaking up the hard clumps of earth in a jar. Invariably on my ceasing to do this, he approached the spot and scratched at it with his front legs. As I have already stated he also approached the female whenever she began to dig. Reversing the conclusion of Montgomery that touch occupies the first place in the senses of sex-recognition and sight the second, I repeat therefore that sight is the only sense of sex-recognition in hunting spiders. After sex has been recognized, courtship begins, and touch is the chief means by which the male excites the female and tests her willingness to accept him.

AN ABERRANT LASIUS [FROM] JAPAN.[1]

WILLIAM MORTON WHEELER.

In a small collection of Japanese ants recently sent me for identification by Professor S. J. Kuwana, of the Imperial Agricultural Experiment Station at Nishigahara, near Tokio, I find a single female specimen of such unusual conformation that I at first supposed it to represent an undescribed genus. On closer examination, however, it proves to be a *Lasius* strikingly different from the females of any of the known species, and suggests two hypotheses for both of which provision will be made in the following paragraphs. The specimen may represent either a new species or merely an aberrant female phase of some one of the known Japanese *Lasii*. The latter supposition will be considered at length in the sequel; the former calls for the following, perhaps merely provisional, taxonomic description:

Lasius spathepus sp. nov. (Fig. 1, *A* and *B*.)

Female (deälated). Length 6 mm.

Head cordate, slightly broader than long, with notched posterior border and rounded, convex posterior corners and sides; convex above; gula concave, with a median longitudinal ridge. Mandibles small, flattened; apparently 5-toothed, with concave external borders. Clypeus depressed, broadly rounded in front, obscurely carinate in the middle. Frontal area obsolescent; frontal groove distinct. Eyes rather large; ocelli small. Antennal scapes broad and compressed, reaching well beyond the posterior corners of the head; funiculi slender, not clavate; all the joints distinctly longer than broad; joints 1–3 more than twice as long as broad; terminal twice as long as the penultimate joint. Thorax much narrower than the head, fully twice as long as broad; mesonotum and scutellum flattened above; epinotum short, rounded above, with the declivity abrupt, straight in profile and longer than the base. Petiole with an erect scale, compressed anteroposteriorly and with its upper margin rather sharp and distinctly notched in the middle. Gaster very short, but little longer than

[1]Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University. No. 22.

broad. Anal papilla prominent. Legs very long; femora, tibiæ and metatarsi dilated and compressed anteroposteriorly; remaining tarsal joints growing successively narrower.

Body and appendages smooth and shining, very finely and inconspicuously punctate. Pleuræ and especially the sides of the epinotum more opaque and somewhat more coarsely punctate. Mandibles opaque, finely and sharply striated.

Hairs yellowish, very short and sparse on the body, denser and more appressed on the flat surfaces of the legs, but absent on the

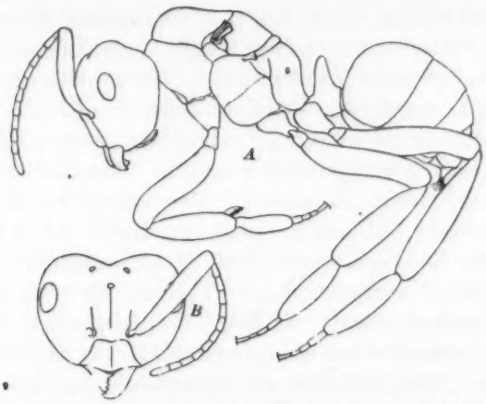


FIG. 1. *A*, *Lasius spathepus* sp. nov., deälated female; *B*, head of same.

sharp dorsal and ventral edges of these appendages. Anterior border of clypeus with a row of short, stout bristles. Border of petiole and posterior edge of each gastric segment with a single row of short hairs. Circlet of anal cilia long and coarse.

Body deep chestnut brown; scapes, legs and articulations of wings paler and more reddish; corners of clypeus and posterior borders of gastric segments sordid yellow.

This female may be at once distinguished from any of the known female *Lasii* by its peculiar heart-shaped head, short gaster and dilated and flattened metatarsi. The last character, in fact, is not met with in any other known ant, except *Melissotarsus*, which Emery regards as an aberrant Ponerine.

The supposition that *L. spathepus* may not be a new species, but merely an extraordinary female form of some one of the well-known Japanese *Lasii*, is supported by the following considerations. Many years ago Walsh[1] described an aberrant female

[1] "On the Genera of Aphidæ found in the United States," *Proc. Ent. Soc. Phila.*, I., No. 9, 1862, pp. 294–311.

Lasius from Illinois as *L. latipes*, and in 1903 McClendon and I[1] showed that this ant has two forms of females: the one described by Walsh and characterized by extremely flat, dilated femora and tibiæ, small, feeble tarsi, strongly clavate antennal scapes, short funicular joints and long, fulvous pilosity; and another of a darker color, with less flattened legs, less clavate scapes, longer funicular joints, longer tarsi and sparser, shorter pilosity. The latter we designated as the α-, the former as the β-female. We found most colonies at the height of the breeding season to contain only β-females, but in three colonies from different localities both forms occurred simultaneously. These observations suggest that *L. spathepus* may be the β-female of some Japanese *Lasius*, which in its worker and male phases shows no departure from the usual generic type of structure. Five *Lasii* are known from Japan, namely, *L. niger* L., *niger alienus* Förster, *myops* Forel, *umbratus* Nyl. and *L. fuliginosus* Latr. All of these are well-known European species and, in all probability, common also throughout temperate Asia.[2] The only one of these species of which *spathepus* could be a β-female is *L. fuliginosus*. I possess males and workers of this species collected by Mr. Hans Sauter in Kanagawa, Japan, and there were three workers in the collection sent me by Professor Kuwana, but as these bear a special number they were probably not taken in the nest containing the *spathepus*. All the Japanese workers and males of *fuliginosus* are indistinguishable from specimens in my collection from several European countries (England, France, Germany, Switzerland, Austria and Russia). In Europe, however, this ant is known to have only one form of female, which is in no respect extraordinary (Fig. 2, *A* and *B*) though it would bear to *spathepus* about the same relation that the α-female of *latipes* bears to the cospecific β-female. Comparison of the figures accompanying this article shows that the head of *spathepus* in its outline is in some respects more like that

[1] "Dimorphic Queens in an American Ant (*Lasius latipes* Walsh)," BIOL. BULL., IV., No. 4, 1903, pp. 149–163.

[2] *L. fuliginosus* is cited by Forel from lower altitudes in the Himalaya ("Les Fourmis de l'Himalaya," *Bull. Soc. Vaud. Sc. Nat.*, 5 ser., XLII., 1906, p. 85). Du Buysson in a paper which I have not seen ("Les fourmis fuligineuses au Japon," *Rev. Ent.*, 1906, pp. 101–102) gives some notes on the occurrence of this ant in Japan.

of the worker *fuliginosus* than the latter is like that of the European female of the species. If *spathepus* is an aberrant female *fuliginosus*, as seems not only possible, but probable, we must therefore assume either that this species in Japan has two females, comparable to the α- and β-females of *latipes*, or that it has only the β-female, while the α-female alone is retained in Europe.

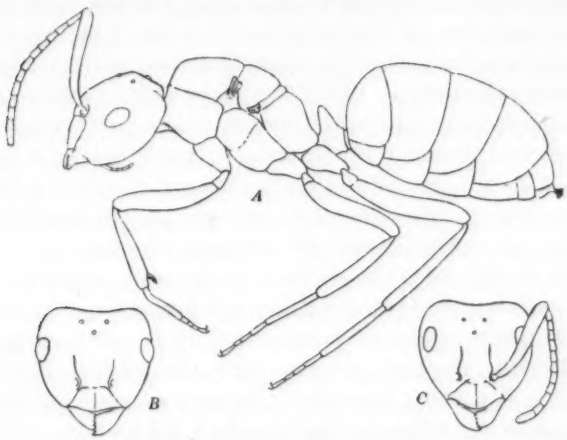


FIG. 2. *A*, *Lasius fuliginosus* Latr., deälated female; *B*, head of same; *C*, head of worker.

Evidently this question can be decided only by exhaustive observations in Japan.

The train of hypotheses suggested by *spathepus* is not terminated at this point. Recent investigations make us look with increasing interest on all aberrant female ants, for it has been found that certain species of *Formica*, *Aphænogaster* and *Bothriomyrmex*-which have females either of unusually small size, glabrous integument, extraordinary color or pilosity, or with unusual morphological characters, also exhibit correlative ethological peculiarities. Such females, during the establishment of their formicaries, are, as a rule, temporary parasites on workers of allied species whose females retain the typical generic characters. The question therefore arises as to whether the aberrant female, which I have called *L. spathepus*, may not be a temporary parasite on some other more common species of *Lasius*. Here the suggestion that *spathepus* may be a β-female of *fuliginosus* receives a little support

from some recent European investigations. Forel[1] long ago showed that this ant is unique among the old world *Lasii* (and the new world species may be included in this statement) in its odor, the great size of its colonies, its habit of foraging in long files in the broad day-light and in constructing carton nests in old tree-trunks. Wasmann[2] has recently called attention to its ability to form new colonies by sending off detachments of queens and workers after the manner of *Formica rufa*. Like *rufa* it also possesses another method of colony formation, namely, through temporary parasitism. Unlike the queens of the common *Lasius niger*, the queen of *fuliginosus*, after fecundation on her marriage flight and on returning to the earth, is unable to start a colony unaided, and if prevented from rejoining the maternal colony or a detachment of workers of her own species, has to seek out a colony of *L. umbratus* and have her young brought up by the workers of this ant. The *umbratus* queen must be killed either by her own workers or by the intrusive *fuliginosus* queen, so that the host species is destined eventually to die off and leave a pure and thriving *fuliginosus* colony. That this method of colony formation is actually adopted by *fuliginosus* queens is clearly indicated by the following observations which have been slowly accumulating during the past few years: In 1908 de Lannoy[3] stated that in 1904 he found at Knoche-sur-Mèr in Belgium a few workers of *L. mixtus* (a subspecies of *umbratus*) living in a large colony of *fuliginosus*, and that in 1906 he found several similar colonies. Emery[4] and Forel[5] interpreted these observations to mean that the queen *fuliginosus* founds her colony with the aid of *umbratus* workers, in a manner analogous to that employed by the North American and European *Formicæ* of the *rufa*, *exsecta* and

[1]"Les Fourmis de la Suisse," *Nouv. Mém. Soc. Helv. Sc. Nat. Zürich*, XXVI., 1874, pp. 1–447, 2 pls.

[2]"Ueber gemischte Kolonien von Lasius-Arten," *Zool. Anzeig.*, XXXV., 1909, pp. 129–141.

[3]"Notes sur le *Lasius niger* et le *Lasius fuliginosus*," *Ann. Soc. Ent. Belg.*, LII., 1908, pp. 47–53.

[4]"Remarques sur les observations de M. de Lannoy touchant l'existence de *L. mixtus* dans les fourmilières de *L. fuliginosus*," *Ann. Soc. Ent. Belg.*, LII., 1908, pp. 182, 183.

[5]"Lettre à la Société Entomologique de Belgique," *Ann. Soc. Ent. Belg.*, LII., 1908, pp. 180, 181.

microgyna groups when they enter nests of *F. fusca* and *incerta*. Wasmann (*loco citato*) accepts the interpretation given by Emery and Forel, and now recalls that he has on several occasions found mixed colonies of *L. umbratus* and *fuliginosus*. Donisthorpe[1] states that in 1897 he recorded the occurrence of a large colony of *fuliginosus* in a hollow tree at Lymington, England, living with what he believed at the time to be *L. flavus* but has since decided must have been *umbratus*. He also says that Crawley has recently found *umbratus* workers in company with *fuliginosus*.

But even this is apparently not the whole story. Crawley[2] found that the queen of *umbratus* may be adopted by a colony of *L. niger*, and Wasmann (*loco citato*) has shown that the former ant is, at least occasionally, a temporary parasite on *niger*, for he found a mixed colony of the two species which could only be interpreted on this supposition. He believes, therefore, that we may have here a case of social hyperparasitism—*umbratus* founding its colonies with the aid of *niger*, and *fuliginosus* with the aid of *umbratus!* In these observations it is, of course, the female of the European *fuliginosus* which exhibits temporary social parasitism, and if *spathepus* is really the β-female of this species, it is also, in all probability, addicted to the same form of parasitism, perhaps on some other species of *Lasius*, although *umbratus*, as I have stated, is known to occur in Japan.

Thus it appears that in the old world the genus *Lasius*, like the genus *Formica*, is made up of two sets of species—one extremely abundant and widely distributed, with queens able to establish their colonies independently, the other rare and sporadic in their occurrence, with queens that require the assistance of workers of other species of the genus when engaged in founding their commonwealths. To the former set belong *L. niger* L. and its various subspecies (*alienus* Förster, *brunneus* Latr., *emarginatus* Fabr., *lasioides* Emery) and *L. flavus;* to the latter *L. umbratus* Nyl., and its subspecies *mixtus* Nyl., *bicornis* Förster and *affinis* Schenck, *L. carniolicus* Mayr and *fuliginosus* Latr. The great rarity of *carniolicus* and the very small size

[1]"On the Founding of Nests by Ants; and a few notes on Myrmecophiles," *Ent. Rec.*, XXII., No. 4, 1910, 4 pp.

[2]*Ent. Month. Mag.*, 1909, p. 94.

of its females point unmistakably to parasitic habits. The same is probably true of *L. crinitus* described by F. Smith[1] and Mayr[2] from Cashmir. Only the female of this species is known and this has long yellow hairs like the North American *Formica ciliata* Mayr and *crinita* Wheeler, which are, in all probability, temporary parasites on varieties of *F. schaufussi* or *fusca*.

In North America, the genus *Lasius*, which embraces the subgenus (*Acanthomyops*) not represented in Eurasia, seems to present a corresponding division of its species into those with independent and those with parasitic queens, although the data on which this assertion is based are at present very meager. Here, too, the forms of *L. niger*, namely the varieties *americanus* Emery and *neoniger* Emery, *L. flavus* var. *nearcticus* Wheeler and *brevicornis* Emery establish their colonies independently. This I can affirm from many observations in the field. The same is true of *L.* (*Acanthomyops*) *claviger* Roger and probably also of *L.* (*A.*) *interjectus* Mayr. But I have never seen any of the females of our *umbratus* forms (*mixtus* var. *aphidicola* Walsh, *subumbratus* Viereck, *minutus* Emery and *speculiventris* Emery) in the act of founding their colonies independently, and it is quite probable that they are temporary parasites on the extremely common *L. americanus*. Equally negative have been my observations on *L* (*A.*) *latipes*, which has the α- and β-females to which I alluded in a preceding paragraph. That this species is a temporary parasite on *L. americanus* is indicated by the fact that near Colebrook, Conn., I found four small mixed colonies.[3] *L.* (*A.*) *murphyi* Forel and *occidentalis* Wheeler, which are closely related to *latipes* and have females covered with singular fulvous hairs, are also very probably to be regarded as parasitic in the early stages of colony formation.

The genus *Lasius*, which comprises some of the commonest and most characteristic ants of the north temperate zone, has never attracted a large number of students, probably because the

[1] "Catalogue of Hymenopterous Insects in the Collection of the British Museum," Pt. VI., Formicidæ, 1858, p. 13.

[2] "Myrmecologische Studien," *Verh. K. K. Zool. bot. Ges. Wien.*, XII., 1862, p. 700.

[3] See Wheeler, "Ants: Their Structure, Development and Behavior," 1910, p. 504, *nota*.

species are in the main subterranean and are so much more monotonous in their habits than the species of *Formica*. Nevertheless, an intensive study of the ethology of the European and North American *Lasii* is bound to bring to light many surprising facts. This is sufficiently indicated in the preceding paragraphs notwithstanding the large amount of conjecture which they contain.

SPERMATOGENESIS OF THE MYRIOPODS.

VI. An Analysis of the Chromosome Group of *Scolopenda heros*.[1]

M. W. BLACKMAN.

During the last few years a number of attempts have been made to analyze the chromosome complex of various animals. These attempts have met with such apparent success in the case of several insects, notably Orthoptera, that I have been led to attempt a similar analysis of the chromosome group in *Scolopendra heros*. Indeed, before the appearance of earlier papers upon this species such an attempt had been made, but it had met with but small success owing, as I now know, to deficiencies in the optical apparatus employed. With the facilities then at my disposal, it was impossible to secure definite clear-cut images of the chromosomes at a magnification greater than 1,500 diameters. As the chromosomes in *Scolopendra heros*, although exceedingly clear-cut and definite in outline, are considerably smaller than in some insects, a greater magnification than 1,500 diameters is necessary if the study is to be at all convincing, either to the investigator or to those reading his report. However, by the use of a Zeiss 2-mm. apochromatic objective and a number 12 compensating ocular, the source of light being a Welsbach mantle, a magnification of 2,300 diameters was obtained with no perceptible loss of definition in the image.

The material used in this study is the same which served as a basis of several previous papers[2] (Blackman :01, :03, :05), the

[1]From the Zoölogical Laboratory, Syracuse University, Syracuse, New York.

[2]Blackman, M. W., :01, "The Spermatogenesis of the Myriapods—I., Notes on the Spermatocytes and Spermatids of *Scolopendra*," *Kans. Univ. Quart.*, Vol. 10, pp. 61–76, pl. 5–7.

Blackman, M. W., :03, "The Spermatogenesis of the Myriapods—II., On the Chromatin in the Spermatocytes of *Scolopendra heros*," Biol. Bull., Vol. 5, pp. 187–217, 22 fig.

Blackman, M. W., :05, "The Spermatogenesis of the Myriapods—III., The Spermatogenesis of *Scolopendra heros*," *Bull. Mus. Comp. Zoöl., Harvard Coll.*, Vol. 48, No. 1., pp. 1–137, 9 pl.

majority of the slides having been mounted nine years, but the stain (Heidenhain's iron-alum hæmatoxylin), except where a portion of the section extends from under the cover-glass, is as perfect as when first mounted.

Sutton,[1] :02, Robertson,[2] :08, and Nowlin,[3] :08, working upon the male cells of Orthoptera have found that the chromosomes during both the spermatogonial and spermatocyte generations may be arranged in a graded series as regards size. In the spermatogonia this series is a double one, the two chromosomes of a given size representing the similar elements derived from the two parents. These similar chromosomes unite during synapsis (Sutton, :02, *op. cit.*), and give rise to the single series characteristic of the spermatocytes. The extreme difference in the size of the chromosomes in the Orthoptera is so marked that it is noticeable at a glance (see Sutton, :02, Fig. 6) and after studying preparations of this material one cannot doubt the accuracy of their observations or deny the strength of their conclusions, that in these forms the chromosomes at any given stage bear a certain size relation to each other, and that this presents strong evidence in support of the theory of the individuality of the chromosomes.

But if we grant these conclusions with regard to the forms studied, does it necessarily follow that these conclusions should be made more general and applied to the chromosomes of all animals? What shall we say as regards the application of this test to the chromosomes of a form in which the difference in size is not so marked or in which the chromosomes all appear of nearly one size? Such is apparently the case in *Scolopendra.* At ordinary magnification there is very little difference in the size of the chromosomes as seen in a metaphase of the first spermatocyte. *Some* difference is to be detected even at a magnification as low as 1,000 diameters, but this is so slight that if size alone be used as a criterion it would seem impossible to distinguish between the chromosomes farther than to say: "This is one of the smaller ones" or "one of the larger ones."

[1]Sutton, W. S., :02, "On the Morphology of the Chromosome group in *Brachystola magna*," BIOL. BULL., Vol. 4, pp. 24–39, 11 fig.

[2]Robertson, W. R. B., :08, "The Chromosome Complex of *Syrbula admirabilis*," *Kansas Univ. Sci. Bull.*, Vol. IV., pp. 275–305, 5 plates.

[3]Nowlin, Nadine, :08, "The Chromosome Complex of *Melanoplus bivittatus* Say," *Kans. Univ. Sci. Bull.*, Vol. IV., pp. 265–271, 2 plates.

But other tests may be applied and have been applied. Baumgartner[1] (:04), made an attempt to distinguish the chromosomes in *Gryllus* by differences in form. He reaches the conclusion that in *Gryllus* certain definite shapes constantly occur and establishes the probability that there is a fixed number of each type. Davis[2] (:08), working upon various Orthoptera reaches the conclusion that "In addition to the difference in volume, the bivalent autosomes (chromosomes) show constant and characteristic differences in form. In general several more or less distinct morphological types can be distinguished, and the members of each type appear to bear a constant numerical relationship to each other."

Robertson, :08 (*op. cit.*), does not consider the shape of the chromosome of first importance in establishing its identity but considers size as the primary characteristic, while shape is secondary and to a certain extent dependent upon size or at least upon the degree of lengthening. The main criticism I wish to make regarding Robertson's conclusions on this point is that in his study of the chromosomes, he has not drawn them from the best view point to establish any characteristic difference in shape. His drawings are all or nearly all of chromosomes as seen in polar view, whereas a view at right angles to the spindle is more satisfactory in determining both the shape of the chromosomes and their relation to the mantle fibers.

In *Scolopendra*, as I have already implied, it is impossible to establish the identity of many of the chromosomes on the basis of size alone. Early in my work, however, after six or eight chromosome groups had been carefully drawn, it became evident that the chromosomes as seen in a lateral view of the metaphase of the first spermatocyte are of several distinct types as regards shape and that the size relation of the chromosomes of each type are such as to make it possible to distinguish the individual chromosomes with some degree of certainty. This, I think, will be apparent from a study of the figures of plates I. and II., although it must be borne in mind that the figures are of course much less satisfactory for this comparison than the actual chromosomes,

[1]Baumgartner, W. J., :04, "Some New Evidences for the Individuality of the Chromosomes," BIOL. BULL., Vol. 8, pp. 1–23, 3 plates.

[2]Davis, H. S., :08, "Spermatogenesis in Acrididæ and Locustidæ," *Bull. Mus. Comp. Zoöl. Harvard Coll.*, Vol. LIII., pp. 59–158, 9 plates.

due to the fact that many of the chromosomes do not lie at right angles to the line of vision and must, therefore, appear foreshortened in an outline drawing.

Before discussing the individual characteristics of the various chromosomes as seen in metaphase, it might be well to give a brief review of their history in the prophase. The spermatocyte chromosomes are seventeen in number. Of these, sixteen are bivalent elements formed by the end to end union of univalent chromosomes during the tetophase of the last spermatogonial division (Blackman, :03, :05, *op. cit.*). The seventeenth element, the accessory chromosome, is univalent in character, being derived directly from a single specialized chromosome, the accessory chromosome of the spermatogonium. The growth period following synapsis is of long duration in *Scolopendra*, and during this period all of the chromosomes are grouped together to form a nucleolus-like structure to which I have given the name karyosphere. While in the karyosphere the chromosomes are so closely aggregated that their individual outlines cannot be distinguished with certainty. They, however, enter the karyosphere as distinct individuals and later arise from it as definite chromatin segments, similar in every respect except for their greater size. These facts would seem to argue for, rather than against, the individuality of the chromosomes during this stage.

In the prophase the chromosomes arising from the karyosphere are typically long, slender threads of granular chromatin, which invariably show near their middle an interruption of the chromatin—this representing the point at which the chromosomes united during synapsis. The two spermatocyte divisions always result in a longitudinal and a cross division of these bivalent elements. The longitudinal division as a rule seems to occur first, although, as we shall see later, this is not invariable, even for the ordinary chromosomes. The cross division or reduction division results in the separation of entire spermatogonical chromosomes, the division occurring at the point at which they united during synapsis. However, although the results of these divisions are the same for all of the chromosomes (with the exceptions to be noted later), the changes through which the tetrad pass in the prophase and the shapes they assume during the pro-

phase and later during the metaphase differ to some extent. As this difference in shape is one characteristic by which we must hope to identify the various chromosomes, occasion may be taken here to describe briefly the processes by which these various forms arise.

What I shall call type A is represented in the text-figure I. The origin and evolution of this type of tetrad is described in sufficient detail in previous papers (Blackman, :03, :05, *op. cit.*); so it will not be necessary to repeat the description in detail.

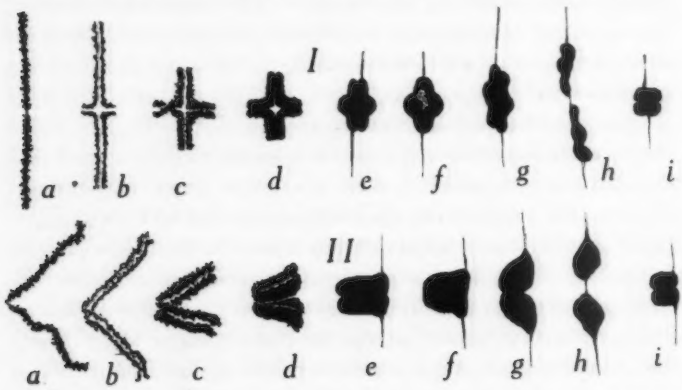


FIG. I. Semi-diagrammatic representation showing the formation and history of the cross-shaped type of tetrads; *a*, bivalent chromatin segment as it appears in the very early prophase; *b*, planes of longitudinal and transverse cleavage established; *c*, *d*, later stage in evolution of prophase tetrad; *e*, *f*, tetrads as seen in early metaphase; *g*, tetrad in act of division, showing the manner in which the component parts glide over each other; *h*, early anaphase showing distortion of halves of tetrad due to their close adhesion; *i*, daughter chromosome in metaphase of second spermatocyte.

FIG. II. Corresponding stages in evolution of the double-V type of tetrad.

I wish, however, to emphasize two points. First the points at the ends of the shorter arms of the cross-like figure (Fig. I, *b*, *c*, *d*) represents the point at which union occurred during synapsis. The attachment of the mantle fibers in the metaphase is *not at this point* as it is said to be in *Syrbula* by Robertson (:08, *op. cit.*), but is at the ends of the longer arms of the cross as shown in Fig. I, *e*, *f*, *g*.

Robertson believes that the attachment of the mantle fibers

coincides with the point of synaptic union of the elements and that each bivalent chromosome during its division undergoes a "change of its long axis from a longitudinal to a transverse direction." This is accomplished by a rotation of the chromatids over each other in such a manner as to result in a longitudinal division of the tetrad in the first spermatocyte. In *Scolopendra*, as I have shown in previous papers (*op. cit.*), no such complicated process occurs in division. The long axis of the tetrad in most cases remains parallel to the line of longitudinal cleavage and in the metaphase the two halves glide over each other during the act of division. As may be seen in the semi-diagrammatic drawings (Fig. I, *g*, *h*) and in several of the chromosomes of this type in the accompanying plates, the two halves of the tetrad seem to adhere rather closely and there is often considerable distortion. In Fig. I, *h*, drawn from my preparations direct it will be seen that the parts of the two daughter chromosomes remaining longest in contact are considerably lengthened and distorted apparently due to the firm adhesion of the two parts.

The second type of spermatocyte chromosome is the "double-V" tetrad described by me in a previous paper (*op. cit.*). This type usually arises from the bivalent chromosomes of the early prophase which are bent at a sharp angle at the point of synapsis. After the longitudinal cleavage of the chromatin thread has occurred the double thread becomes shorter and thicker, resulting in the double-V-shaped structure shown in Fig. II, *c*. There is at all times a very apparent interruption of the chromatin at the angle of each thread (point of synapsis), and it is at this point that the cross division occurs later. In the late prophase there is a still further condensation of the chromatin and shortening of the thread, resulting in the closer apposition of the ends of the threads farthest from the point of synapsis, resulting in a chromosome of the shape shown in Fig. II, *e*, *f*. At the time of the formation of the spindle the mantle fibers come to be attached to the distal ends (ends farthest from point of synapsis) of the tetrad in such a manner (Fig. II, *e*, *f*, *g*) that the chromosome is divided along its longitudinal axis. In this type also, the two halves of the chromosome seem to adhere closely and to divide reluctantly (Fig. II, *g*, *h*, also Figs. 6, *i*, and 17, *j*).

The chromosomes of the third type arise from thread-like structures similar to those from which type A and B arise. This thread may be either approximately straight, or it may be curved slightly in various ways, but is never bent at a sharp angle at the point of synapsis. The filament undergoes a longitudinal cleavage just as with the other types. The two resulting threads, as a usual thing, lie parallel to each other (Fig. III, *b*, *c*) but in some

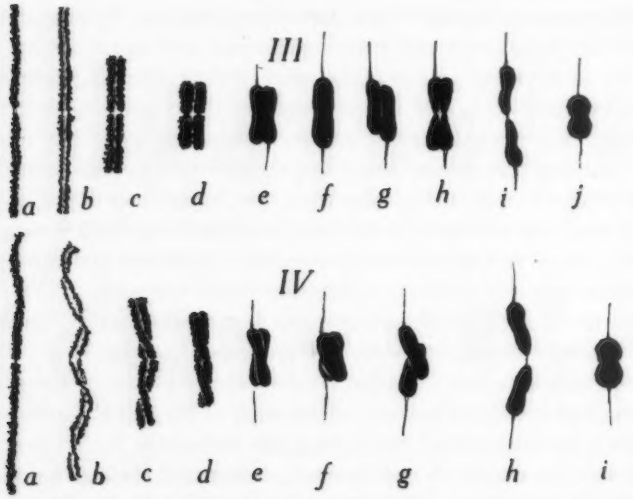


FIG. III. Evolution of the double-rod-shaped tetrads. *a*, bivalent chromatin segment; *b*, *c*, *d*, formation of tetrad; *e*, *f*, tetrads as seen in early stages of the spindle; *g*, *i*, ordinary tetrad in two stages of longitudinal division; *h*, rod-shaped tetrad apparently in act of transverse division; *j*, dyad as seen in metaphase (secondary spermatocyte).

FIG. IV. Variation of double-rod-shaped tetrad. In early prophase the double chromatin segment is often twisted as shown in *b*. The shortening of chromatin thread results in less and less twisting; so that the two parts of the metaphase chromosome merely overlie each other at an angle or are only partially wrapped about each other.

cases they are twisted about each other, so as to form a rope-like structure (Fig. IV, *b*, *c*). In such cases the resulting chromosome has a somewhat different shape. In this type of chromosome the tetrad resulting appears rod-shaped or double rod-shaped depending upon the angle from which the structure is viewed.

After the planes of longitudinal and cross division are established, the further changes involve merely a shortening and condensation of the entire structure. In Fig. III, *c*, *d*, two stages in this process are shown, both cleavages being very evident. Later, as the condensation continues these planes of cleavage are obscured to some extent and usually the only evidence we have of the longitudinal cleavage is a very definite notch at each end of the chromosome, while the plane of cross division is usually shown merely by a slightly lessened diameter of the tetrad near its middle (Fig. III, *d*, *e*, *f*). In some cases, however (Fig. 3, *g*, *h*), the planes of longitudinal and transverse cleavage may be seen very distinctly even in the metaphase (Fig. 4, N, 13, *n*, 16, *n*, etc.). In metakinesis a longitudinal division is accomplished by a gliding apart of the two halves of the tetrad (Fig. III, *g*, *i*) in a manner essentially similar to the division of the cruciform tetrads.

In a number of the cells studied there seems to be one of the chromosomes of this type, which presents quite a different appearance during the act of division. A constriction appears in this chromosome at about the middle point (the plane of transverse cleavage). This is so pronounced that there is in many cases (dependent on the stage of division) a partial or even a complete interruption of the chromatic material at this point (Figs. 3, N, 4, N, 9, N, 10, N, etc.). From a careful study of this chromosome in many cells, the conclusion seems inevitable that this one tetrad undergoes a transverse division, while the rest of the chromosomes are dividing longitudinally.

A variation of the rod-shaped tetrad is shown in text-fig. IV. Here after the longitudinal cleavage has been established, the two threads, instead of lying side by side, are twisted about each other in such a way as to form a rope-like structure. The resulting metaphase chromosome differs somewhat in appearance from others of this type, although in all essentials it is identical. As the threads shorten the twisting gradually becomes less and less pronounced (Fig. IV, *b*, *c*, *d*, *e*) until in the completed chromosomes there is only a slight twisting and usually the two parts merely overlie each other at an angle. The division accomplished by the first maturation mitosis is a longitudinal one.

Besides these three types of ordinary chromosomes all of which

are bivalent there is one element which is univalent in character, being derived directly from a single spermatogonial chromosome. This is the accessory chromosome and in the metaphase can always be distinguished by its characteristic shape, and especially by the fact that it is connected by mantle fibers to only one pole of the spindle. There is usually no indication of the plane of division in the accessory chromosomes at this time, although in the prophase a longitudinal constriction is often shown.

The chromosomes of *Scolopendra heros* are seventeen in number, sixteen of which are bivalent, while one is univalent. The sixteen bivalent chromosomes undergo longitudinal and transverse divisions during the prophase and at the beginning of the metaphase are of several different types as regards shape. After studying a large number of metaphases of both the large and small (Blackman, :05) type of spermatocytes it has been found to be a rule that in each cell the chromosomes of the several types bear a definite and constant numerical relationship to each other. This fact can best be appreciated by referring to the accompanying plates. It will be seen at a glance that the cruciform tetrads which have been described above as type A are in all cases six in number. Furthermore, among the chromosomes of this typical form a definite size relation exists which makes it possible to arrange the cross-shaped chromosomes in a graded series on the basis of bulk. To be sure, the difference in size is not so striking as that existing between the largest and smallest chromosomes in some insect material, but I believe is as great as the difference in size between adjacent chromosomes of the graded series in insects. It is perhaps unnecessary to explain that the *actual* size difference is in many cases greater than appears in the camera lucida drawings, owing to the fact that some of the chromosomes are foreshortened on account of the angle from which they are viewed. For this reason, the drawings are much less convincing than the preparations.

The shape of the chromosomes, aside from showing an apparent modification due to the angle of vision, actually does vary considerably but each of the group of six chromosomes in question are always reducible to the cruciform tetrad described as type A. The variations in shape have to do only with the degree to which

the short arm of the cross is drawn out and to apparent differences incident to the angle of vision. When the short arm of the cross is drawn out but little the tetrad approaches the rod-shaped chromosomes of type C. Then, too, the cruciform tetrads vary in shape in different stages of the metaphase. The limbs of the cross are more likely to be of nearly equal size in the early spindle stages than when division has actually begun. As the chromosomes even in the same equatorial plate, are not all in exactly the same stage of division at the same time this factor should receive consideration.

An attempt was made to learn whether any of the chromosomes constantly either lag behind or precede the others in division. It was found that chromosome A, the largest of the cross-shaped tetrads, shows a tendency to lag somewhat behind the others of this type. In many of the chromosome groups this is very evident. In some cells it is not yet oriented in the characteristic manner in the equatorial plate at a time when some of the others have begun to show the characteristic change in shape incident to the gliding apart of their component elements. This can be seen by comparing chromosome A with other chromosomes of similar type in Figs. 1, 3, 4, etc. Then, too, this chromosome often presents a less clear-cut outline than do the others, approaching the granular condition characteristic of the prophase.

Of the chromosomes of type B there are five present in the metaphase of *Scolopendra heros*. This is the type of chromosome which shows the characteristic double-V shape in the prophase. The difference in shape between the tetrads of this type and the cross-shaped elements is usually quite striking. Even more characteristic of this type is the attachment of the mantle fibers and the orientation of the chromosomes in the equatorial plate. As seen in side view, the chromosome is more or less rectangular in shape, but one end is usually wider than the other and to the angles of this end the mantle fibers are attached. The chromosome usually lies with this end toward the center of the spindle, while the free end (that to which the mantle fibers do not attach) extends outward. This free end is often notched and this notch indicates the plane of longitudinal division. In end view (Figs. 1, *I*, 6, *J*, etc.) the appearance is not so characteristic in the draw-

ings, although in the preparations there is no difficulty in recognizing the true shape of the chromosome, the apparent difference in shape being due to the view-point from which it is seen.

The five chromosomes of type B form a graded series as regards size, just as with those of type A. The largest one is very perceptibly greater than the smallest, and the intermediate ones differ in size to such an extent that there is usually little difficulty in assigning them to their proper place in the series. No individual of this type shows any constant precocity or tardiness in division, although in some cells one or more of them are farther along than the others (Figs. 6, *I*, 17, *J*).

The rod-shaped tetrads (type C) are five in number in *Scolopendra heros*. They show the same constancy in size relation as do the other types, and may be readily arranged in a graded series. Usually one or more of this type of chromosome show the component parts overlapping each other at an angle or partially wrapped around each other, indicating that they arise from the twisted threads often seen in the prophase and already described. However, these are not constant in occurrence and this condition seems to depend largely upon chance.

A fact which has proved rather puzzling was brought out by a careful study of the various chromosomes of this type. While it cannot be doubted that four of the rod-shaped chromosomes divide longitudinally in the first spermatocyte division, the fifth tetrad of this shape apparently divides transversely. In all cases in which this element is well advanced in the metaphase there is a very evident constriction at its middle point, and in some cases this amounts to a nearly complete interruption of the chromatic material. This is especially evident in Figs. 3, 4, 10, chromosome *N*. Indeed, it seems hardly possible to escape the conclusion that at the same time the other fifteen bivalent chromosomes are undergoing longitudinal division, this one element is divided reductionally.

This, however, is no less to be expected than is the behavior of the accessory chromosome in this same division. It differs from the other chromosomes in being univalent (*i. e.*, it has no synaptic mate), while the rest are bivalent. After the formation of the spindle it lies among the other chromosomes and is scarcely distinguishable from the rod-shaped ones aside from the fact that

it is connected by mantle fibers to only one pole of the spindle. It is not divided by the first spermatocyte division but passes to one pole entire. Thus the result is in a sense similar to a reductional division, the two cells differing as regards the distribution of this element. It has no synaptic mate and is, therefore, distributed to only one of the resulting cells (Figs. 19, 20).

It is evident that the unequal distribution of the accessory chromosomes produces two sorts of secondary spermatocytes—one half having only the sixteen ordinary chromosomes and one half having the accessory chromosome in addition. By the second spermatocyte division, the accessory chromosome is divided and as it occurs in but half of the second spermatocytes it is distributed to only half of the spermatids, thus giving rise to a dimorphism among the spermatids and spermatozoa. The significance of this dimorphism has been discussed by a number of investigators—McClung[1] (:02), Wilson[2] (:06), Stevens[3] (:08, :08*a*, :09) Boring[4] (:07) and others—and, as I have nothing new in the way of observations to offer it would appear hardly profitable to consider the subject in detail. I believe, however, that when the chromosomes of the female germ cells of *Scolopendra* are studied it will be found that these are thirty-four in number in the ovogonia, and that the following fertilization formulæ of Wilson (:06, *op. cit.*) will hold good for this species:

$$\text{Egg } \frac{N}{2} + \text{Spermatozoön } \frac{N}{2} \begin{pmatrix} \text{including} \\ \text{accessory} \end{pmatrix} = N \text{ (female).}$$

$$\text{Egg } \frac{N}{2} + \text{Spermatozoön } \frac{N}{2} - 1 \begin{pmatrix} \text{accessory} \\ \text{lacking} \end{pmatrix} = N - 1 \text{ (male).}$$

[1]McClung, C. E., :02, "The Accessory Chromosome—Sex Determinant?" BIOL. BULL., Vol. 3, pp. 43–84.

[2]Wilson, E. B., :06, "Studies on Chromosomes—III., The Sexual Differences of the Chromosome Group in Hemiptera, with some Considerations on the Determination and Inheritance of Sex," *Journ. Exp. Zoöl.*, Vol. III., pp. 1–40, 6 fig.

[3]Stevens, N. M., :08, "A Study of the Germ Cells of Certain Diptera with Reference to the Heterochromosomes and the Phenomena of Synapsis," *Journ. Exper. Zoöl.*, Vol. V., pp. 359–374, 4 plates.

Stevens, N. M., :08*a*, "The Chromosomes in *Diabrotica vittata*, etc.," *Journ. Exper. Zoöl.*, Vol. V., pp. 453–469, 3 plates.

Stevens, N. M., :09, "Further studies on the Chromosomes of Coleoptera," *Journ. Exper. Zoöl.*, Vol. VI., pp. 101–113. 4 plates.

[4]Boring, Alice M., :07, "A Study of the Spermatogenesis of Twenty-two Species of the Membracidæ, Jassidæ, Cercopidæ and Fulgoridæ," *Journ. Exp. Zoöl.*, Vol. IV., pp. 469–512, 9 plates.

In *Scolopendra* (Blackman, :05, *op. cit.*) there are two distinct types of spermatocytes readily divisible on the basis of size. Those characterized by the larger size are about twice the average diameter of the smaller ones and vary from them in behavior in the two maturation divisions. But this variation in behavior concerns the achromatic structures of the cell and seems to be due to the much greater amount of cytoplasmic and archoplasmic material present in the larger cells. It is extremely interesting to note that as regards the behavior of the chromosomes these two sizes of spermatocytes are essentially identical. Indeed, the chromosome groups of the small cells differ in no respect from those of the large type. The elements are no smaller than in many of the large cells and present the same constancy in form and the same size relations characteristic of the large type of cells. Figs. 16, 17, 18 represent the chromosome groups of the small type of spermatocytes. Many more were carefully studied and drawn and all show the same characteristic shapes and size relations typical of the spermatocyte chromosomes. In fact, the only reason more of these were not used is that they are not so desirable for study owing to the difficulty in drawing them due to their closer crowding in the metaphase.

The shape of the daughter chromosomes as they move apart to the poles in the anaphase of the first spermatocyte mitosis is quite characteristically different for the different types of chromosomes (Figs. I, II, IV, *h*, III, *i*). Those resulting from the division of cross-shaped tetrads have the three lobed appearance shown in Fig. I, *h*. The daughter chromosomes resulting from the division of the double-V-shaped tetrads have the shape shown in Fig. II, *h*, and are essentially single-V-shaped chromosomes, as is shown at a later stage. Those resulting from the division of the double-rod tetrads, as they move toward the poles, have the form of single rods, slightly constricted near the middle. I have been unable to identify positively the division products of the tetrad which undergoes its reduction division in the first mitosis, but in several anaphases six of the daughter chromosomes of each group are V-shaped, and it is probable that the sixth one of this shape is the chromosome in question. This is rendered more certain by the observation that in these cells there are but

four of the rod-shaped chromosomes exclusive of the accessory, which, of course, is present in but one of the chromosome groups.

The chromosome groups, as seen in the metaphase of the two cells derived from one primary spermatocyte are shown in Plate II., Figs. 19, 20. The most striking fact to be observed is the absence of the accessory chromosome in one of the cells. Even a superficial examination, however, shows that in shape and relation to the mantle fibers the chromosomes are of several different types, and that these characteristics coincide with what would be expected from a study of their earlier history. The chromosomes derived from the cross-shaped tetrads have altered their shape considerably since last seen in the anaphase of the first maturation division. They are much shorter and thicker and are now bilobed bodies—the constriction between the lobes representing the plane of transverse cleavage. The attachment of the mantle fibers seems to be at no particular point but may be at any part of each flattened end of the dyad.

The structural peculiarities of the chromosomes derived from the double-V-shaped tetrads are much more characteristic. In shape the dyads of this type resemble those just described to some extent, but, except in size, bear a more striking resemblance to the double-V-shaped chromosomes from which they are derived. One end, usually the one nearest the center of the equatorial plate, is broader than the other, and the entire structure very evidently corresponds to the single-V-shaped chromosomes of the first spermatocyte anaphase. The mantle fibers are always attached to the broader end of the dyad, this fact being even more characteristic than the shape. The appearance of these chromosomes while in the act of division might lead one to believe that the resulting division is a longitudinal one, but such a conclusion would ignore entirely the previous history of this type of chromosomes during the prophase and metaphase of the first maturation division.

Chromosomes L, M, O and P, in the second spermatocyte (Figs. 19, 20) are the product of the longitudinal division of the rod-shaped tetrads. They are dumbbell-shaped dyads with a mantle fiber attached to each end. The constriction at the middle of each represents the plane of transverse division. Chro-

mosome N shows a considerably different shape, corresponding to its different history. It is shorter and in one plane broader than the other dyads derived from the double-rod-shaped tetrads of the first spermatocyte. It has been already shown that chromosome N of the first spermatocyte probably undergoes a transverse division while the other tetrads are dividing longitudinally, and we would, therefore, expect the products of this division to present a different appearance from the other dyads. As a matter of fact, it is of quite a different shape from the others derived from the double-rod tetrads.

The differences in size between the various chromosomes of the different types is, of course, only half as great in the second spermatocyte, as it is in the first spermatocyte, and therefore there is not such certainty in identifying the various individuals of the different types. But the same size ratio seems to exist and the chromosomes of the different types can readily be arranged in a graded series as regards size, just as in the first spermatocyte.

It has been shown by this study that the chromosomes of *Scolopendra heros* cannot be considered as ephemeral structures, which have one appearance in one cell and present an entirely different form in another cell of similar history. Any study except a very superficial one must lead to an entirely different conclusion. By a study of many hundreds of cells in various stages of mitosis it has been found that the number of chromosomes in the primary spermatocytes is absolutely constant and invariable. Furthermore, these chromosomes show other characteristics, which speak very strongly for their individuality. The ordinary chromosomes are divisible into three types on the basis of the shape they assume in the prophase and metaphase of the first maturation division, and in their relation to the mantle fibers of the spindle. The individuals of each type of structure are invariably of the same number and in all favorable cases each chromosome of a given type is distinguishable from the others of a similar shape by a difference in size.

In addition, several of the chromosomes possess certain individual peculiarities aside from shape and size, which serve

further to characterize them. One of the cross-shaped tetrads is tardy in orienting itself in the plane of the spindle. Another chromosome, one of the rod-shaped ones, shows a much more striking and fundamental peculiarity, in that it differs from all of the rest of the bivalent chromosomes in the plane of its divisions in the first and second spermatocytic mitoses. The accessory chromosome shows still more striking peculiarities, differing from the others in its origin, valence, behavior in the prophase, relation to the mantle fibers of the spindle and in its distribution to but one half of the resulting cells.

All of the facts enumerated above offer evidence which seems conclusive that the chromosomes of *Scolopendra heros*, during the spermatocyte stages at least, must be considered as distinct entities, each one possessing certain well defined peculiarities which are as characteristic for any given chromosome of the spermatocytes as are the peculiarities of a species of animals. I believe that eventually in many animals it will be possible to make this statement still broader and to demonstrate the continuity of the individual elements from cell generation to cell generation. We will, then, be able to say that, while in different cell generations or different conditions of cell activity, the appearance and behavior of any given chromosome may be quite different, just as is true of many animals in different stages of their existence, yet in a similar cell generation any particular chromosome will present the same appearance and will behave in the same manner. I believe that the condition described above is true in *Scolopendra heros* but several facts conspire to make it impossible of physical demonstration.

These difficulties are mechanical difficulties and have to do with the small size of the chromosomes in the spermatogonial stages and the close aggregation of these elements in the karyosphere of the growth period. The difficulties due to the small size of the chromosomes in the spermatogonial stages appears insurmountable, and the only evidences of individuality which they present have to do with their absolute constancy in number, and with the very characteristic behavior of the accessory—it being the only element which can be identified at all stages. We might reason from this that because one of the elements

displays unmistakable individuality all of the chromosomes possess individuality. This argument has been made in other cases and, while the continuity of the accessory chromosome *does* offer valuable evidence in support of the individual continuity of the chromosomes in general, it cannot be said to establish the truth of the general theory.

The difficulty of establishing the individuality of the chromosomes during the growth period would seem fully as great as during the spermatogonial period. During all the stages in which the karyosphere exists the chromosomes are so densely aggregated that it is impossible to distinguish the separate elements. But even at this time it is possible in favorable cases to distinguish the accessory chromosomes and to discern the outlines of some of the other elements. Furthermore, as I have shown in previous papers (Blackman, :05, *op. cit.*) the chromosomes enter the karyosphere as distinct bivalent elements, and at the end of the growth period arise from it as distinct chromatic segments of the same number and character as in the earlier stage.

The chromatin segments entering the karyosphere are bivalent threads formed by the union and subsequent diffusion of two spermatogonial chromosomes. The point of union of synapsis shows very plainly as a distinct interruption of the chromatin granules near the middle of the segment, the interval being bridged by linin fibers. In favorable sections of the karyosphere (*i. e.*, those in which the stain has been sufficiently extracted) it is seen that this body is made up of a number of chromatin segments closely massed about the accessory. The chromosomes on leaving the karyosphere are of the same structure as when they entered, are of the same number and in appearance differ from those of an earlier period in size only. In fact, the larger spermatocyte chromosomes possess nearly as great a bulk as the entire chromosome group of the spermatogonium, this immense increase in size being accompanied by a growth of other parts of the cell, which is proportionally even greater.

It would appear then, that during certain stages of the spermatogenesis of *Scolopendra* it is possible to demonstrate absolutely that each chromosome is a distinct unit characterized by certain definite and constant peculiarities and that the continuity of

each element can be traced from the early prophase of the first spermatocyte to the anaphase of the second maturation division. In other words, it is evident that during this very important period of their history the chromosomes show complete individuality. In other stages, namely, in the spermatogonia and during the growth period, it cannot be claimed that the continuity of the chromosomes is actually demonstrated in *Scolopendra*, although evidence strongly supporting such a view undoubtedly exists.

Summary.

The chromosome group of the primary spermatocytes of *Scolopendra heros* is made up of sixteen bivalent chromosomes (tetrads) and one univalent chromosome (dyad), the accessory chromosome.

The chromosomes show such constancy in shape in the prophase and metaphase of the primary spermatocytes, and in their relation to the mantle fibers of the first maturation spindle, that they seem naturally to group themselves under four distinct types. These may be designated respectively, as the cross-shaped tetrads, the double-V-shaped tetrads, the rod-shaped tetrads, and a single-rod-shaped dyad.

The cross-shaped tetrads are six in number and may be arranged in a graded series as regards size, the difference in bulk being sufficiently great to allow the individual chromosomes of this type to be distinguished. One of the chromosomes of this type (the largest one) can furthermore often be identified by its tendency to lag behind the others during the early metaphase.

Five of the tetrads are of the double-V shape. The individuals of this type also may be distinguished by differences in bulk.

The rod-shaped tetrads are present to the number of five. These show constant size relations and may readily be arranged in a graded series as regards magnitude. One of the tetrads of this type differs from the others in the form it assumes during actual division. It seems to divide transversely, while the others are dividing longitudinally.

The accessory chromosome is univalent and passes to one of the secondary spermatocytes without division. During the

metaphase it is connected by mantle fibers to only one pole of the spindle.

As a result of the first spermatocyte mitosis fifteen of the tetrads are divided longitudinally (equationally), while the one remaining tetrad divides transversely (reductionally). The failure of the accessory chromosome to divide is, also, in effect a reductional division.

During the later stages of the first maturation division and during the metaphase of the second spermatocyte, it is possible to distinguish the daughter chromosomes derived from the several types of tetrads, by their shape and their relations to the mantle fibers. The individuals of the various types show the same size ratio as exists between the chromosomes of the first spermatocyte, although, of course, the actual difference in bulk is but half as great.

The above results seem to establish as a fact, or at least as a very strong probability, that the chromosomes of *Scolopendra heros* are distinct and definite individuals, which, under similar circumstances, *i. e.*, in the same cell generation, show a remarkable constancy in form, relative size, and in their attachment to the mantle fibers. This constancy of form, size and behavior, affords a strong argument in favor of the theory of the individuality of the chromosomes in this species in particular and adds support to the evidence derived from the study of other forms, to the general application of the theory.

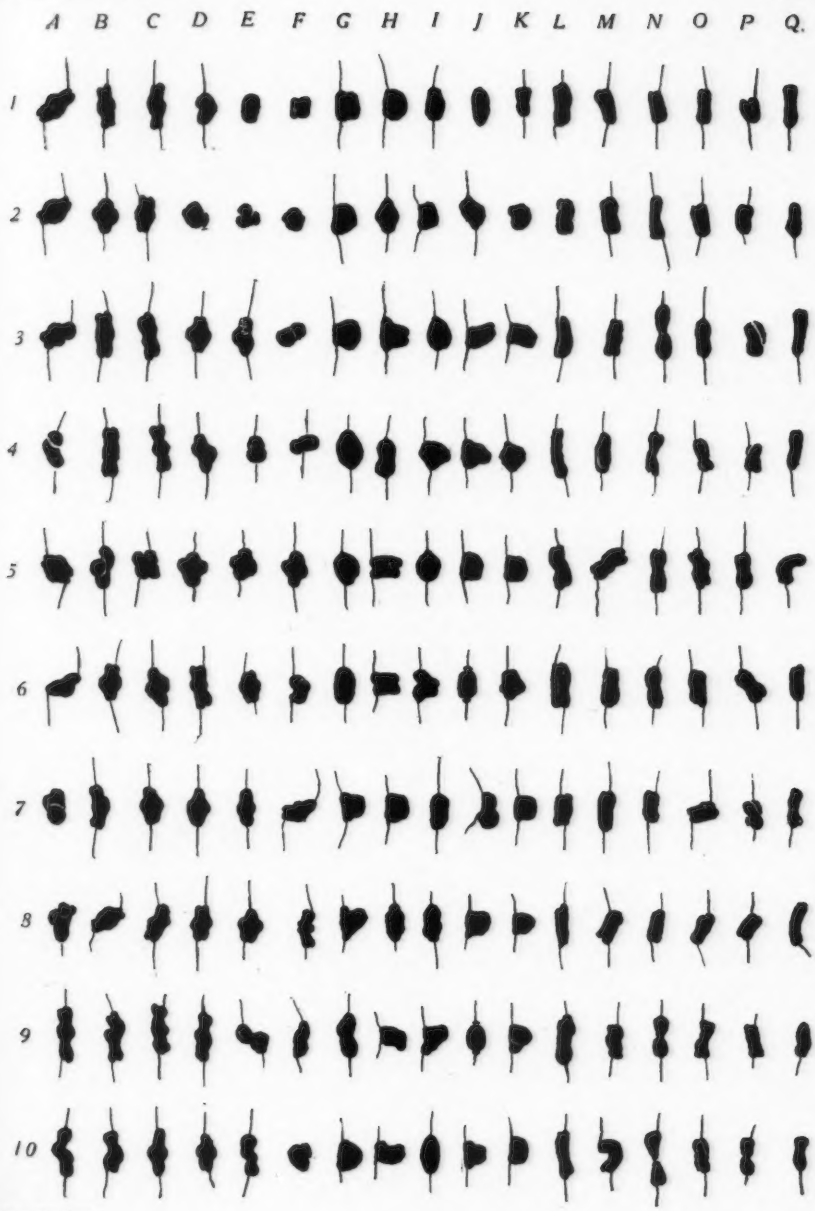
LABORATORY OF ZOÖLOGY,
SYRACUSE UNIVERSITY,
April 11, 1910.

Explanation of Plate I.

All drawings were made by the author with the aid of a camera lucida. The optical equipment consisted of a Zeiss apochromatic objective of 2 mm. focus and a number 12 compensating ocular, the source of the light being a Welsbach mantle The original magnification was 2,300 diameters and the drawings were reduced one fifth in reproduction, making the final magnification 1,840 diameters.

The seventeen chromosomes arranged in each horizontal row represent the chromosomes of a single cell, as seen in a side view of the spindle in the metaphase. They are arranged as follows: the six which show the characteristic cruciform shape comprise the first six of each row and are lettered A, B, C, D, E and F. Those showing the double-V shape—five in number—are lettered G, H, I, J and K. Those corresponding to the double-rod type of structure are lettered L, M, N, O and P. The seventeenth and last chromosome in each row is the accessory and is distinguished by the letter Q. The individuals of each type of structure are further arranged in a graded series as regards size, the largest first, etc.

Figs. 1–15 represent the chromosome groups in the metaphase of the large type of first spermatocytes.



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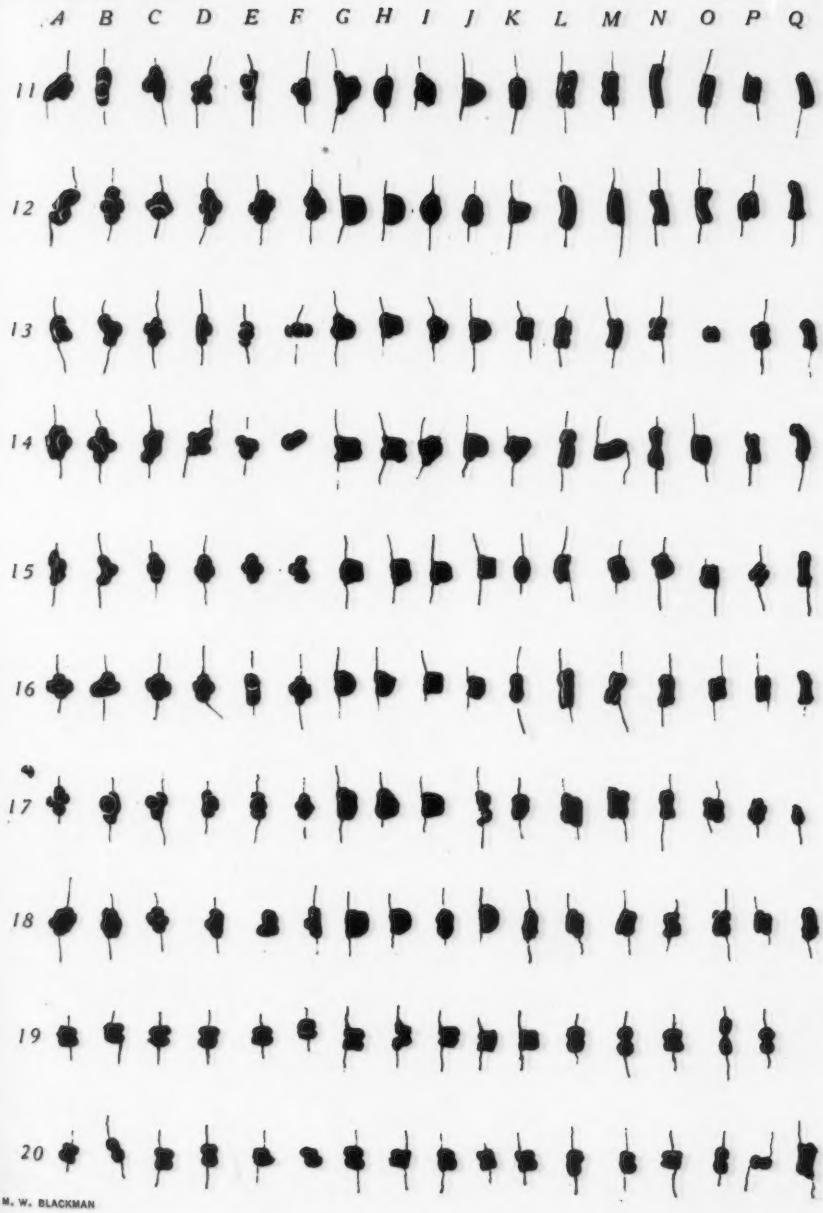
Explanation of Plate II.

Figs. 16–18 represent the chromosomes of the small type of spermatocyte in a similar stage.

Figs. 19 and 20 represent the chromosomes of the two second spermatocytes, derived from one primary spermatocyte. The individual chromosomes are arranged so as to correspond in position to the parent chromosomes as seen in the other figures. As will be seen, in one cell the accessory chromosome is not present, it being distributed to only half of the secondary spermatocytes.



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